



# Agilent MassHunter Qualitative Data Analysis

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Stephen Harnos

MassHunter Qualitative Analysis

Chromatogram Functions

# MassHunter Qualitative Analysis Software B.07.00

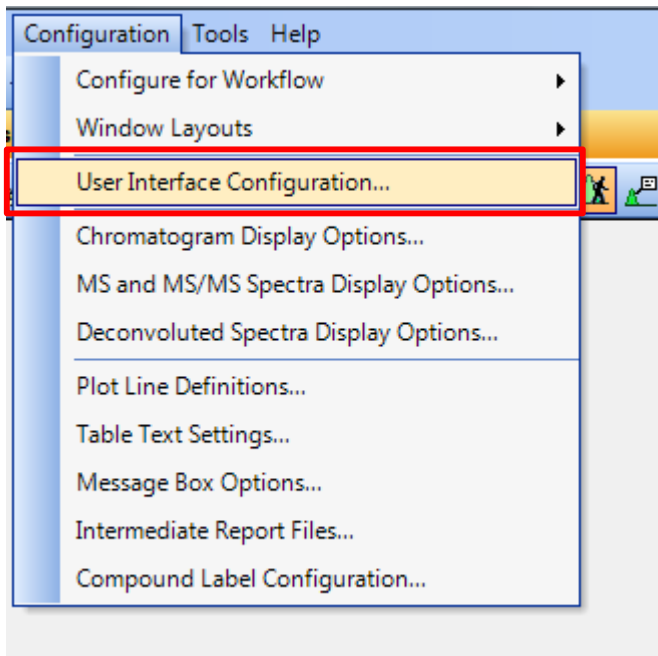
## Topics

- User Interface Configuration
- User Workflows
- Views
  - Navigator
  - Compound Details
- Methods
  - Unified Method Concepts
  - Method Explorer
  - Method Editor
- Working with Chromatograms
  - Anchoring and Scaling
  - Chromatogram Functions
  - Integrators
- Training Resources

# MassHunter Qual - Configurable Software

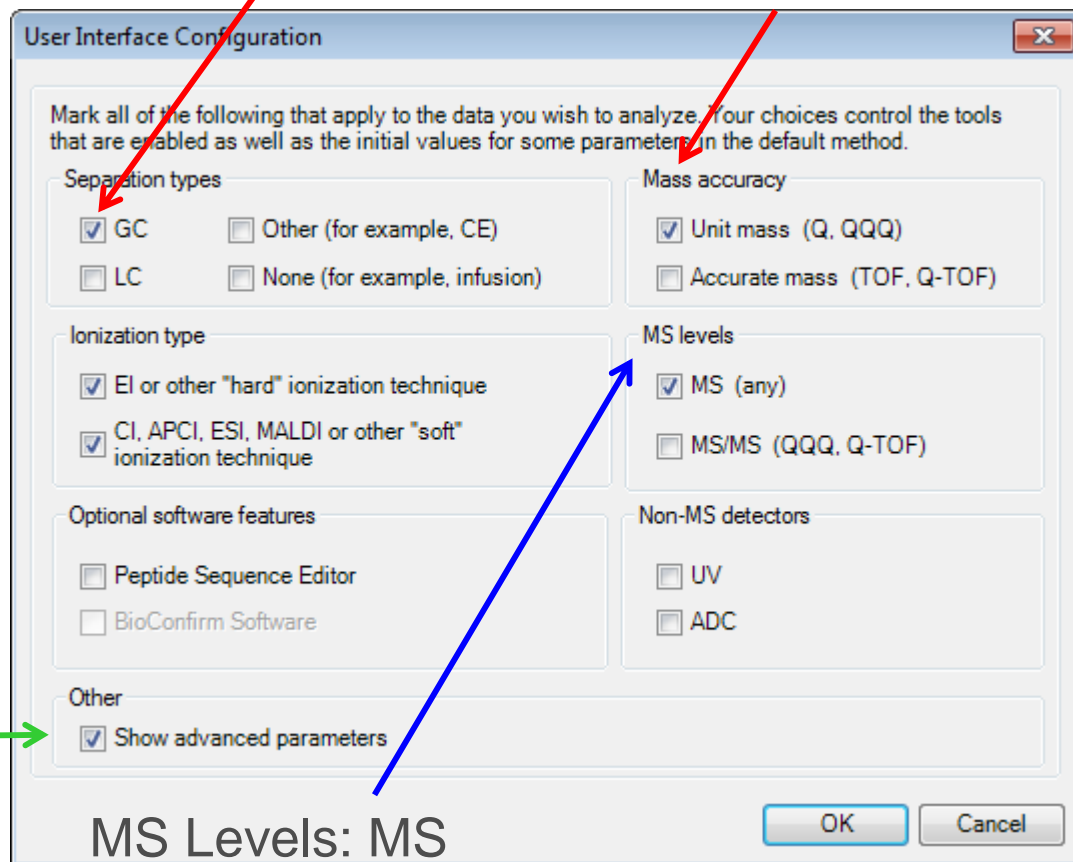
- One program for many instruments and types of data.
  - Single Quad (LC & GC) Unit resolution, Scan, SIM data
  - Triple Quad (LC & GC) Unit Resolution Scan, SIM, MRM (MS/MS) data
  - TOF (LC) High resolution, scan data
  - Q-TOF (LC & GC) High resolution MS/MS data
- Many software features can be used by all data types but many are only useful for a particular instrument type.
- MassHunter Qual **MUST** be configured to reduce complexity and hide unneeded and potentially misused features.
- Even when properly configured some features and parameters for MS/MS and accurate mass are still visible, ignore and avoid them.

# User Interface Configuration



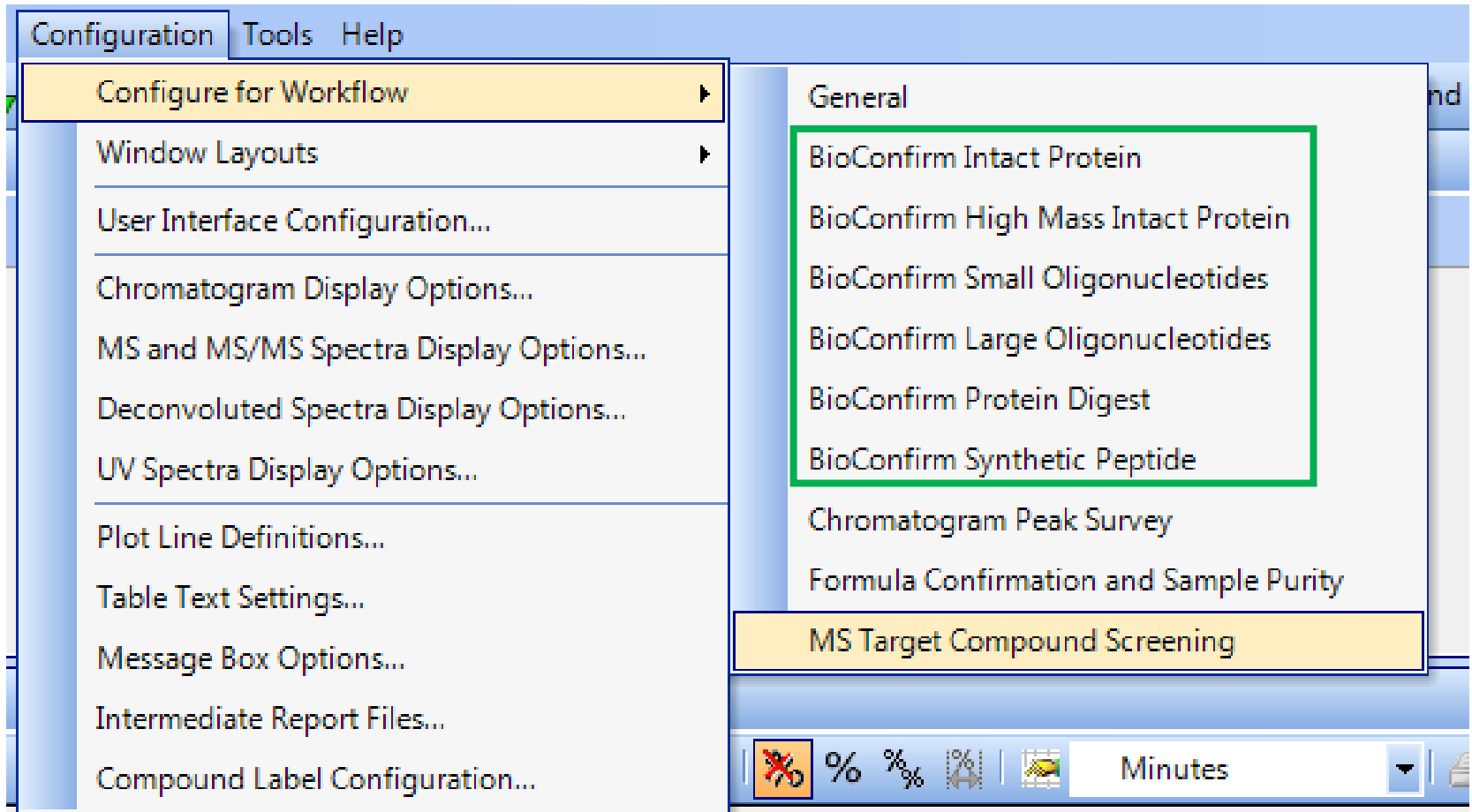
Separation types  
(Check GC or LC)

Unit Mass (Q, QQQ)  
Accurate Mass  
(TOF, QTOF)



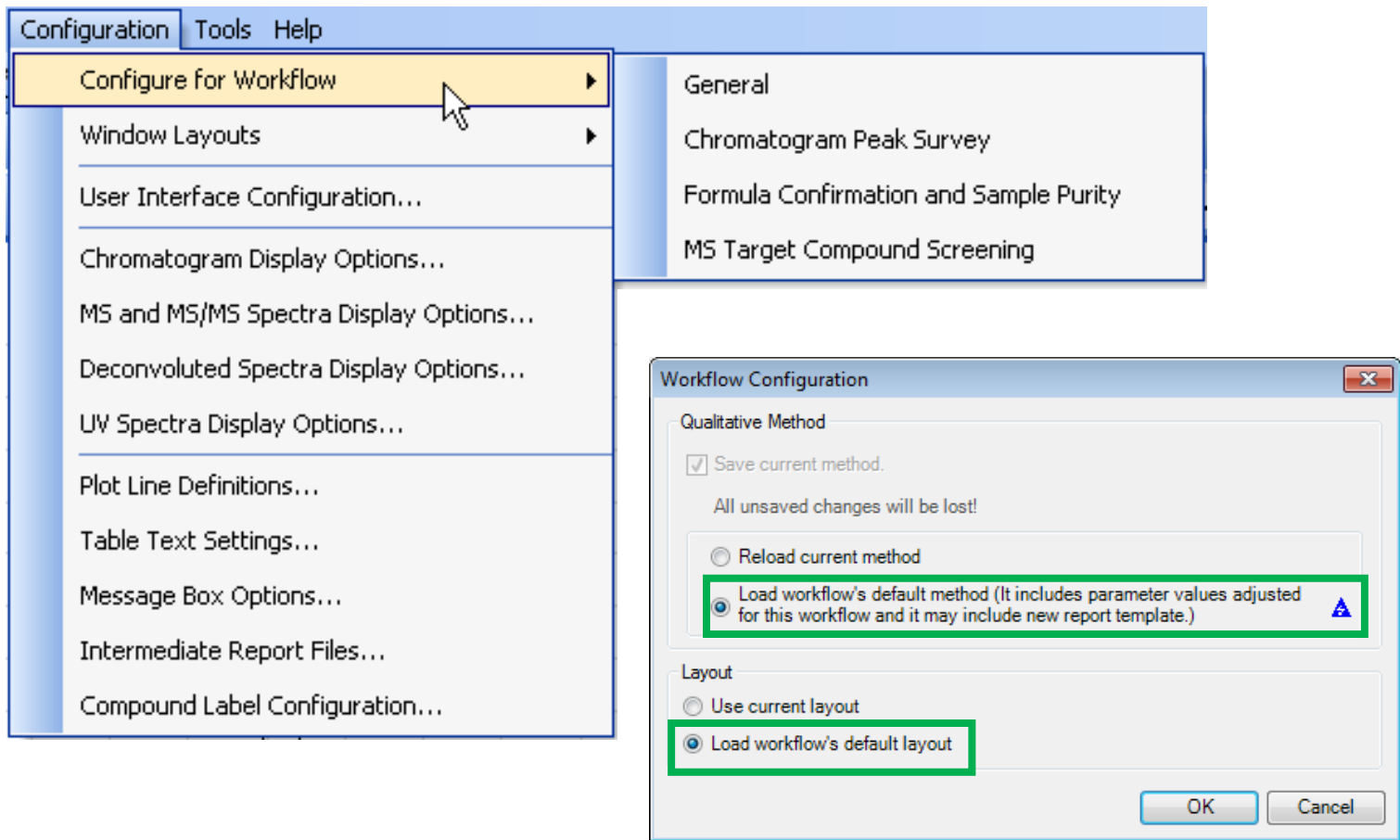
Check Show Advanced Parameters

# MassHunter Qualitative Analysis Workflows



**Depends Upon Software Loaded and Configuration Selected**

# Configure for Workflow

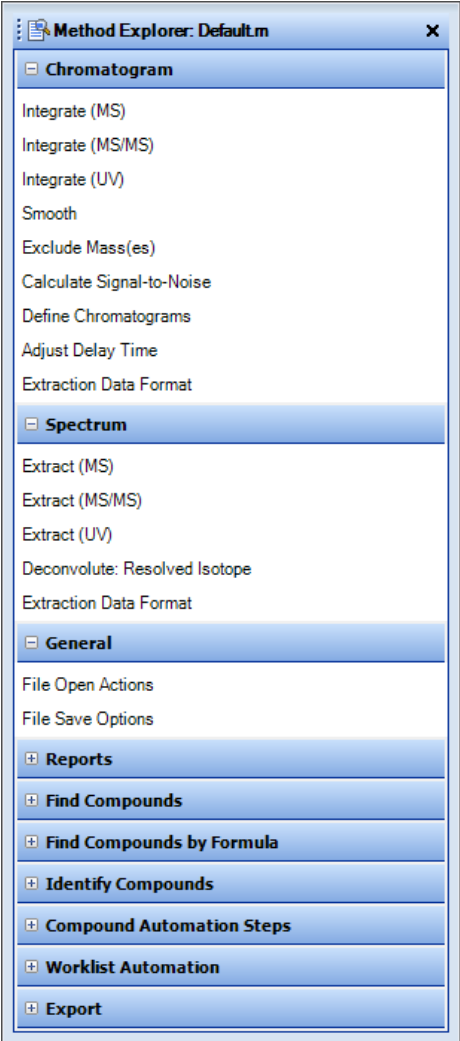


**Configuration Changes Graphics, Table, and Method Layouts.**

**Tip: Load workflow's default method and default layout.**

# Chromatogram Peak Survey Workflow

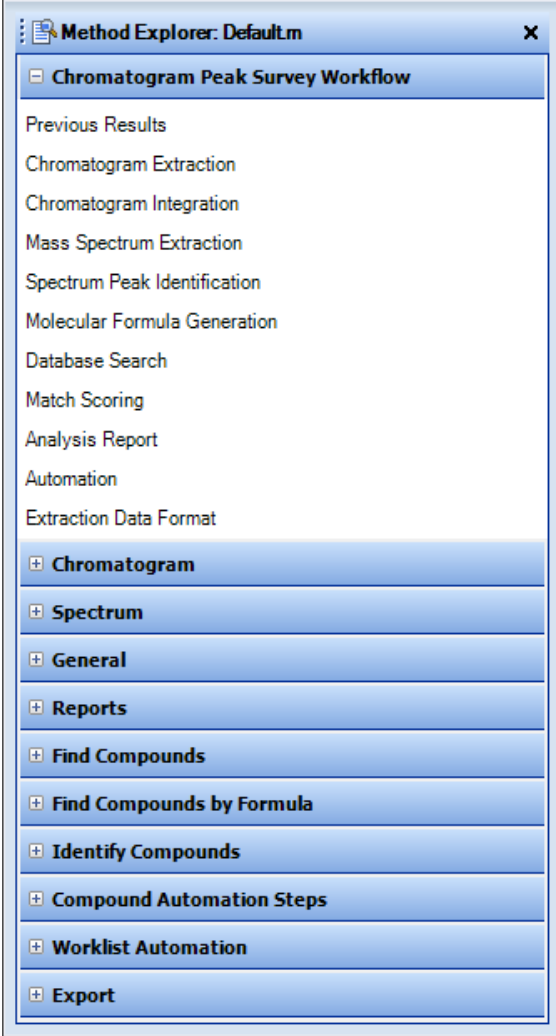
## General



Modify settings for chromatogram.

Modify settings for spectral extraction.

## Chromatogram Peak Survey



Specified workflow added.

# MS Target Compound Screening Workflow

## General

## MS Target Compound Screening Workflow

Method Explorer: Default.m

- Chromatogram
  - Integrate (MS)
  - Integrate (MS/MS)
  - Integrate (UV)
  - Smooth
  - Exclude Mass(es)
  - Calculate Signal-to-Noise
  - Define Chromatograms
  - Adjust Delay Time
  - Extraction Data Format
- Spectrum
  - Extract (MS)
  - Extract (MS/MS)
  - Extract (UV)
  - Deconvolute: Resolved Isotope
  - Extraction Data Format
- General
- File Open Actions
- File Save Options
- Reports
- Find Compounds
- Find Compounds by Formula
- Identify Compounds
- Compound Automation Steps
- Worklist Automation
- Export



Method Explorer: Screening-Default.m

- MS Target Compound Screening Workflow
  - Extraction Data Format
  - Chromatograms
  - Mass Spectra
  - Find by Molecular Feature
  - Find by Formula
  - Identify by Database Search
  - Identify by Library Search
  - Identify by Formula Generation
  - Match Scoring
  - Compound Report
  - Automation
- Chromatogram
- Spectrum
- General
- Reports
- Find Compounds
- Find Compounds by Formula
- Identify Compounds
- Compound Automation Steps
- Worklist Automation
- Export

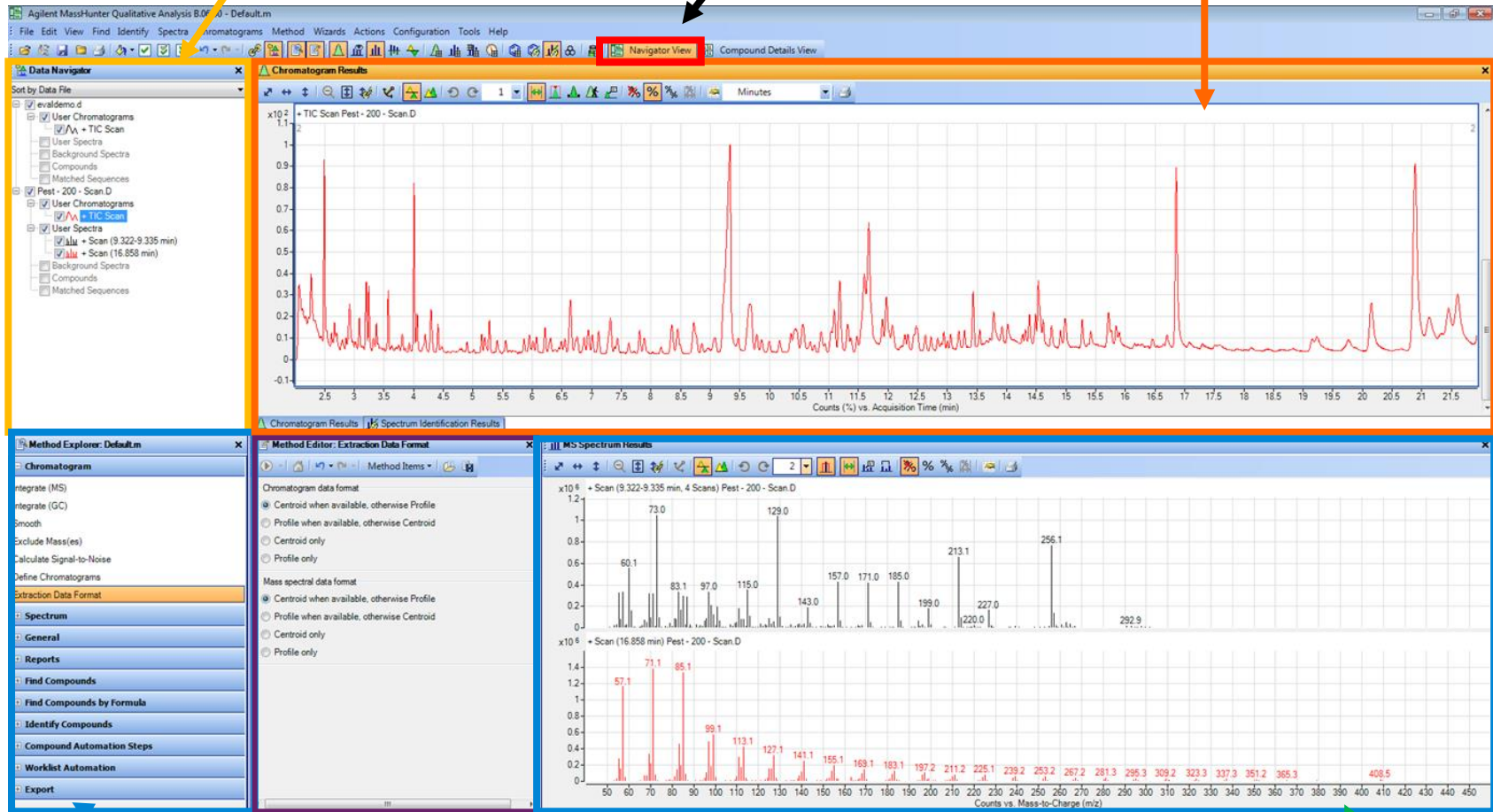


# Navigator View

Data Navigator

Navigator View

Chromatogram Results



Method Explorer

Method Editor

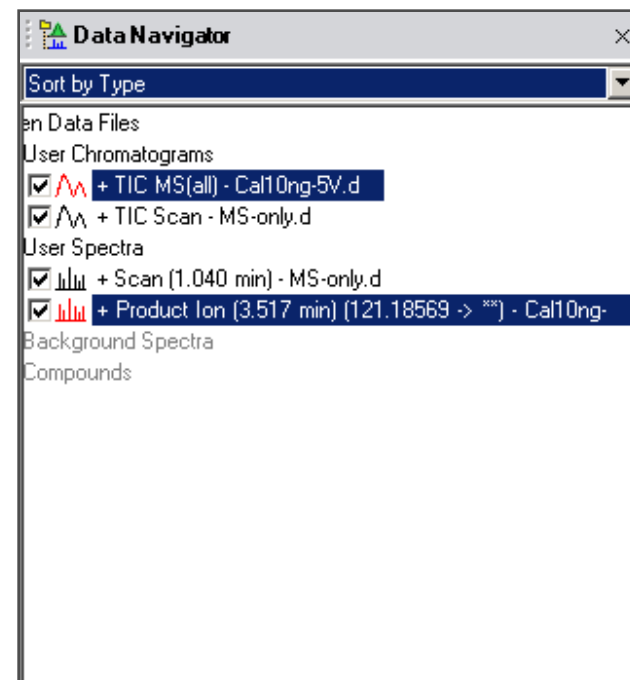
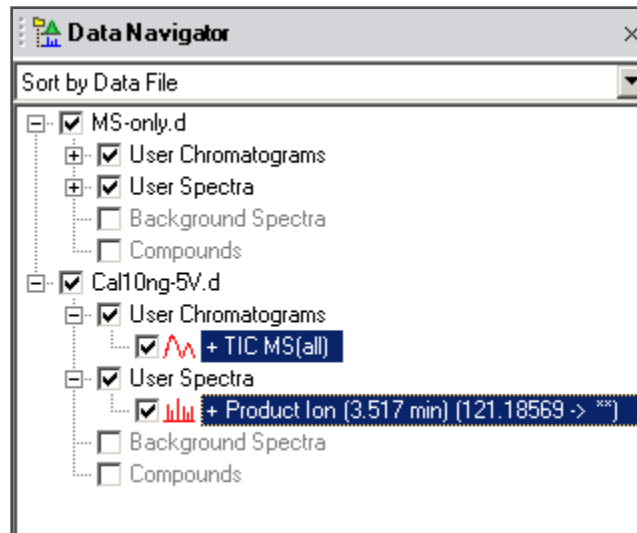
Spectrum Results

# Data Navigator

The Data Navigator pane shows the data files which are loaded into Qualitative Analysis.

The user can selectively display the information associated with a data file (i.e. chromatograms, spectra, compounds) by selecting/deselecting a checkbox.

In the top drop-down, the user can choose to sort by Data File or Type (i.e. User Chromatogram, etc.)



# Compound Details View

Compound Details View

Compound List

The screenshot displays the Agilent MassHunter Qualitative Analysis B.06.00 interface. The main window is titled 'Compound List' and contains a table of identified compounds. A red arrow points to the 'Compound Details View' tab, and a blue arrow points to the 'Compound List' tab. Below the main table, there are three sub-windows:

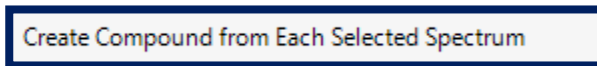
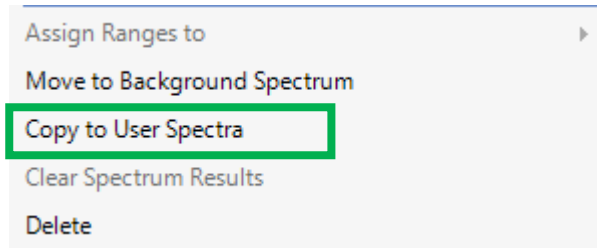
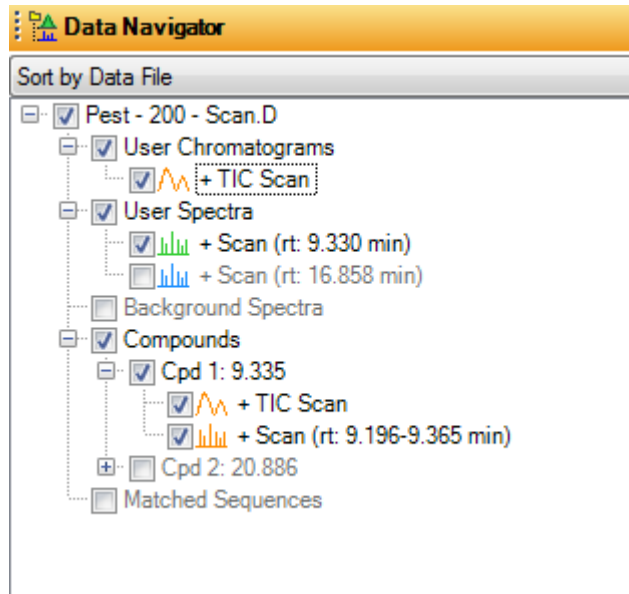
- Compound Chromatogram Results:** Shows a chromatogram with a single prominent peak at 5.892 minutes, labeled 'Cpd 76: alpha-Ketopantoic acid'. The x-axis is 'Counts vs. Acquisition Time (min)' and the y-axis is 'Minutes'.
- Compound MS Spectrum Results:** Shows two mass spectra for 'Cpd 76: alpha-Ketopantoic acid'. The top spectrum is an MFE Spectrum with a base peak at 169.0470 m/z. The bottom spectrum is an ESI Scan with a base peak at 101.0602 m/z. The x-axis is 'Counts vs. Mass-to-Charge (m/z)'.
- Compound Fragment Spectrum Results:** Shows a message: 'No Fragment Spectrum available for this compound.'

Compound Chromatogram Results

Compound Fragment Spectrum Results

Compound MS Spectrum Results

# Definitions



User Spectra are mass spectrum that the user creates.

Compounds are generated by one of the 'Find by' algorithms. Compounds are generated by the software.

User Spectra and Compounds are readily interchangeable through the context menu (right click on the User or Compound Spectrum in the MS Spectrum Results window).

# Expose or Hide Windows as Needed

Menu

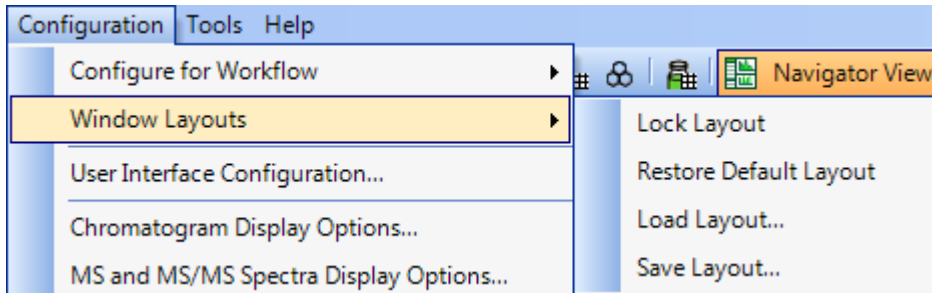
Toolbar

The screenshot displays the Agilent MassHunter Qualitative Analysis B.06.00 - Default.m interface. The 'View' menu is open, showing options such as Data Navigator, Method Explorer, Method Editor, Chromatogram Results, Spectrum Preview, MS Spectrum Results, Difference Results, Deconvolution Mirror Plot, Integration Peak List, MS Spectrum Peak List 1, MS Spectrum Peak List 2, MS Actuals, Compound List, Compound Identification Results, Spectrum Identification Results, Structure Viewer, Sample Information, Status Bar, and Linked Navigation. The 'Define Chromatograms' option is highlighted. The main workspace contains three windows: 'Chromatogram Results' showing a Total Ion Chromatogram (TIC) for 'pest-20ppm.D' with peaks labeled at retention times (e.g., 4.190, 4.704, 5.057, 5.401, 5.961, 6.766, 7.138, 7.628, 7.811, 7.972, 8.414, 8.772, 8.998, 9.243, 10.519); 'Method Editor: Define Chromatograms' showing a list of defined chromatograms including 'BPC (all) MS (Cycle-summed)'; and 'MS Spectrum Results' showing two mass spectra for scans 4.181-4.200 min and 4.694-4.713 min, with peaks labeled at m/z values (e.g., 60.1, 95.0, 130.0, 164.9, 202.9, 236.9, 271.8, 63.1, 89.1, 148.1, 165.1, 205.9).

# Docking & Undocking Windows

The screenshot displays the Agilent MassHunter Qualitative Analysis B.06.00 interface. The main window is titled "Chromatogram Results" and shows a Total Ion Chromatogram (TIC) for "pest-20ppm.D". The x-axis represents time in minutes, ranging from approximately 4 to 10.5. Several peaks are labeled with their retention times: 4.190, 4.704, 5.057, 5.401, 5.961, 6.766, 7.138, 7.811, 7.928, 7.972, 8.414, 8.772, 8.998, 9.243, and 10.519. A red box highlights a cluster of peaks around 7 minutes, and another red box highlights a peak at 7.138 minutes. A "Spectrum Preview" window is docked over the chromatogram, showing a mass spectrum for the selected peak. The x-axis is labeled "m/z of interest" and ranges from 50 to 450. The y-axis is labeled "Counts vs. Mass-to-Charge (m/z)". The spectrum shows several peaks, with the most prominent one at m/z 236.9. Other labeled peaks include 4.9, 202.9, 271.8, 5.1, 63.1, 89.1, 148.1, and 205.9. The "Method Editor: Define Chromatograms" window is also visible, showing the "BPC (all) MS (Cycle-summed)" method. The "Data Navigator" on the left shows a tree view of the data files, including "User Chromatograms" and "User Spectra". The "Method Explorer: Default.m" window is also visible, showing various processing options like "Integrate (MS)", "Integrate (GC)", "Smooth", "Exclude Mass(es)", "Calculate Signal-to-Noise", "Define Chromatograms", "Extraction Data Format", "Spectrum", "General", and "Reports". Red arrows indicate the docking and undocking of the "Spectrum Preview" window. The "Spectrum Preview" window is docked over the chromatogram, and the "Method Editor: Define Chromatograms" window is docked over the "Spectrum Preview" window. The "Data Navigator" window is docked on the left side of the main window. The "Method Explorer: Default.m" window is docked at the bottom left of the main window. The "Chromatogram Results" window is the main window, and the "Spectrum Preview" window is a floating window that can be docked or undocked. The "Method Editor: Define Chromatograms" window is a floating window that can be docked or undocked. The "Data Navigator" window is a floating window that can be docked or undocked. The "Method Explorer: Default.m" window is a floating window that can be docked or undocked.

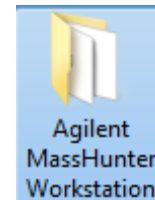
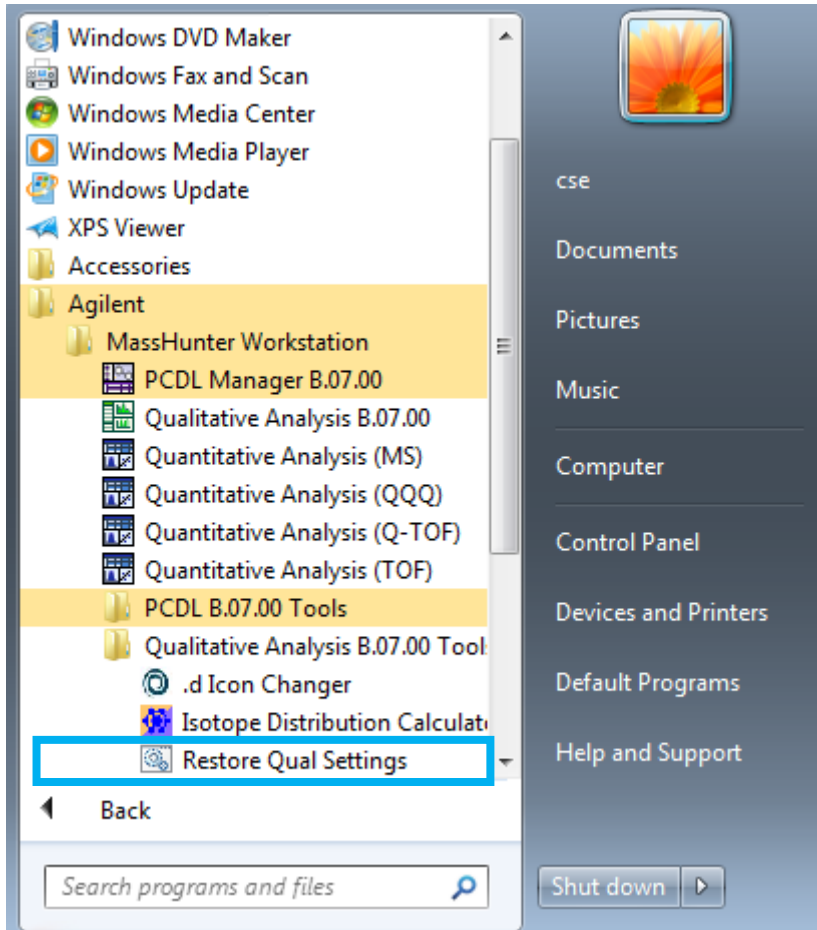
# Restore Default Layout



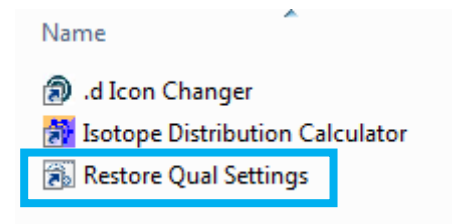
- Complicated windows layouts can be restored to default layout.
- Preferred layouts can be saved and loaded.
- Layouts can be locked.

# Restore Qual Setting

This may be a useful tool to restore the Qualitative Analysis settings if a configuration problem is suspected.

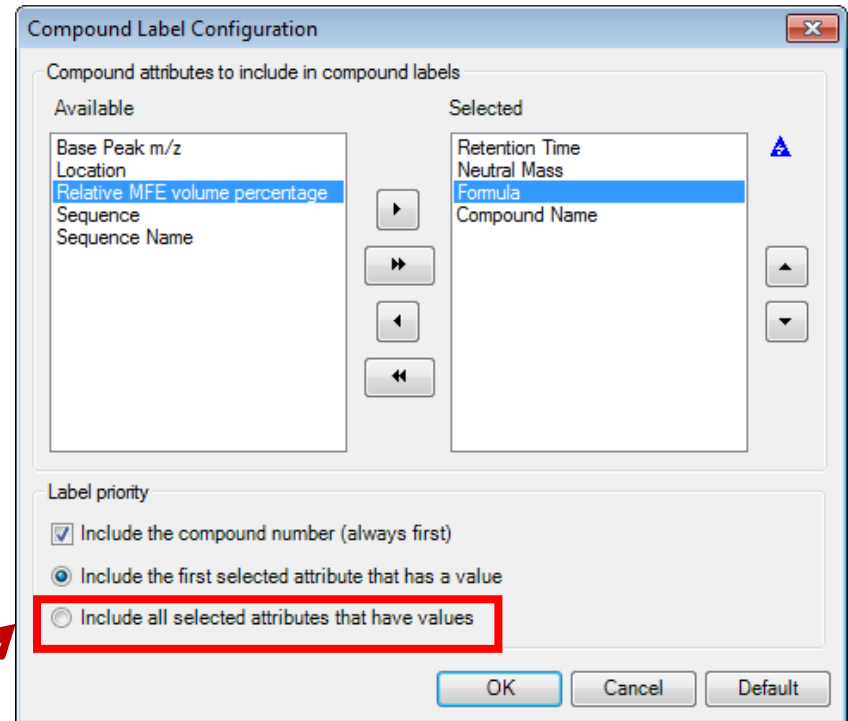
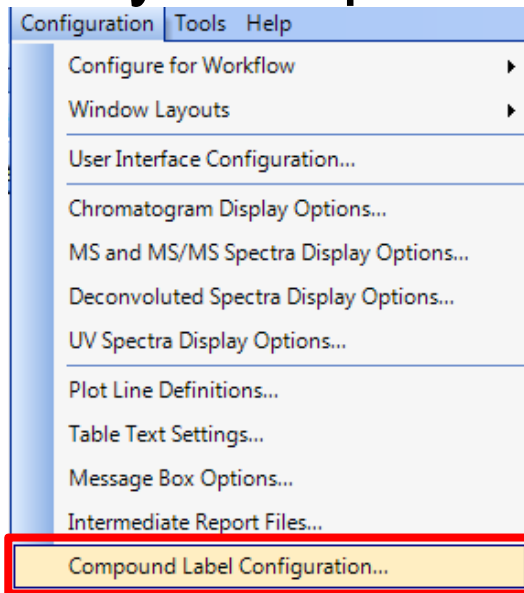


Or Desktop folder





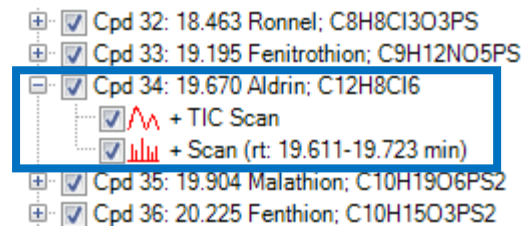
# Specify Compound Label Configuration



Configuration > Compound Label Configuration

Tip: Select Include all selected attributes that have values.

In this example, compounds shows the compound number, RT, compound and formula.



# Compounds Labels Display in Data Navigator

Sort by Data File

<input checked="" type="checkbox"/>	Compounds
<input checked="" type="checkbox"/>	Cpd 13: 1.020 169.0848; C7 H11 N3 O2; C7 H11 N3 O2; N(pai)-Methyl-L-histidine
<input checked="" type="checkbox"/>	Cpd 14: 1.039 103.0996; C5 H13 N O
<input checked="" type="checkbox"/>	Cpd 16: 1.068 161.1047; C7 H15 N O3
<input checked="" type="checkbox"/>	Cpd 17: 1.114 113.0586; C4 H7 N3 O; C4 H7 N3 O; Creatinine
<input checked="" type="checkbox"/>	Cpd 20: 1.146 115.0992; C6 H13 N O
<input checked="" type="checkbox"/>	Cpd 23: 1.193 85.0892; C5 H11 N
<input checked="" type="checkbox"/>	Cpd 24: 1.195 140.0581; C6 H8 N2 O2; C6 H8 N2 O2; Ethyl-imidazole carboxylate
<input checked="" type="checkbox"/>	Cpd 25: 1.215 170.0687; C7 H10 N2 O3; C7 H10 N2 O3; 2,3,4-Trihydroxybenzylhydrazide
<input checked="" type="checkbox"/>	Cpd 26: 1.232 228.1104; C10 H16 N2 O4
<input checked="" type="checkbox"/>	Cpd 27: 1.278 143.0945; C7 H13 N O2; C7 H13 N O2; Triparanol
<input checked="" type="checkbox"/>	Cpd 28: 1.318 137.0476; C7 H7 N O2; C7 H7 N O2; 2-Pyridylacetic acid
<input checked="" type="checkbox"/>	Cpd 29: 1.328 175.0955; C6 H13 N3 O3; C6 H13 N3 O3; Citrulline
<input checked="" type="checkbox"/>	Cpd 30: 1.346 202.1316; C9 H18 N2 O3; C9 H18 N2 O3; Ala Ile
<input checked="" type="checkbox"/>	Cpd 32: 1.420 85.0895; C5 H11 N
<input checked="" type="checkbox"/>	Cpd 33: 1.450 203.1164; C9 H17 N O4; C9 H17 N O4; L-Glutamic acid n-butyl ester
<input checked="" type="checkbox"/>	Cpd 34: 1.464 159.1257; C8 H17 N O2; C8 H17 N O2; DL-2-Amino-octanoic acid
<input checked="" type="checkbox"/>	Cpd 35: 1.471 211.0948; C9 H13 N3 O3; C9 H13 N3 O3; Zalcitabine
<input checked="" type="checkbox"/>	Cpd 37: 1.499 145.0857; C5 H11 N3 O2; C5 H11 N3 O2; 4-(diaminomethylideneamino)butanoic acid
<input checked="" type="checkbox"/>	Cpd 38: 1.539 216.1468; C10 H20 N2 O3; C10 H20 N2 O3; Val Val
<input checked="" type="checkbox"/>	Cpd 39: 1.613 268.1168; C11 H16 N4 O4; C11 H16 N4 O4; Isobutylglycine
<input checked="" type="checkbox"/>	Cpd 40: 1.623 244.0697; C9 H12 N2 O6; C9 H12 N2 O6; Uridine
<input checked="" type="checkbox"/>	Cpd 42: 1.646 192.0265; C6 H8 O7; C6 H8 O7; 2,3-Dioxogulonic acid
<input checked="" type="checkbox"/>	Cpd 43: 1.647 137.9956; C6 H2 O4
<input checked="" type="checkbox"/>	Cpd 44: 1.648 174.0159; C6 H6 O6; C6 H6 O6; Dehydroascorbic acid
<input checked="" type="checkbox"/>	Cpd 45: 1.655 180.0643; C7 H8 N4 O2; C7 H8 N4 O2; Theobromine
<input checked="" type="checkbox"/>	Cpd 46: 1.660 228.1470; C11 H20 N2 O3; C11 H20 N2 O3; Leu Pro
<input checked="" type="checkbox"/>	Cpd 47: 1.667 216.1223; C8 H16 N4 O3
<input checked="" type="checkbox"/>	Cpd 48: 1.685 169.0844; C7 H11 N3 O2; C7 H11 N3 O2; N(pai)-Methyl-L-histidine
<input checked="" type="checkbox"/>	Cpd 49: 1.685 141.0791; C7 H11 N O2; C7 H11 N O2; Ethosuximide
<input checked="" type="checkbox"/>	Cpd 51: 1.775 129.0425; C5 H7 N O3; C5 H7 N O3; Pyroglutamic acid
<input checked="" type="checkbox"/>	Cpd 52: 1.776 158.1415; C8 H18 N2 O

# Open Data Files

File Edit View Find Identify Spectra Chromatograms Method Wizards Actions Configuration Tools Help

Open Data File... Ctrl+O

Refresh Data File Save Results Ctrl+S Close Data File Close All Print Export Import Compound... Exit

Chromatogram Results

Open Data File

Look in: Pest

- pest-0.02ppm.D
- pest-0.05ppm.D
- pest-0.1ppm.D
- pest-0.2ppm.D
- pest-0.5ppm.D
- pest-01ppm.D
- pest-02ppm.D
- pest-05ppm.D
- pest-10ppm.D
- pest-20ppm.D
- QuantResults

File name: "pest-0.05ppm.D" "pest-0.1ppm.D" "pest-0.2ppm.D"

Files of type: Data Files (\*.d)

Options

- Load worklist method
- Load results method
- Use current method
- Load result data
- Run 'File Open' actions from selected method

Sample Information

Sample Name :  
User Name :  
Sample Position :  
Description :

Method Explorer: Default.m

- Chromatogram
  - Integrate (MS)
  - Integrate (GC)
  - Smooth
  - Exclude Mass(es)
  - Calculate Signal-to-Noise
  - Define Chromatograms
  - Extraction Data Format
- Spectrum
  - Extract (MS)

Fragmenter: Any Ionization: Any

Select multiple files at once for batch analysis, then click **Open**.

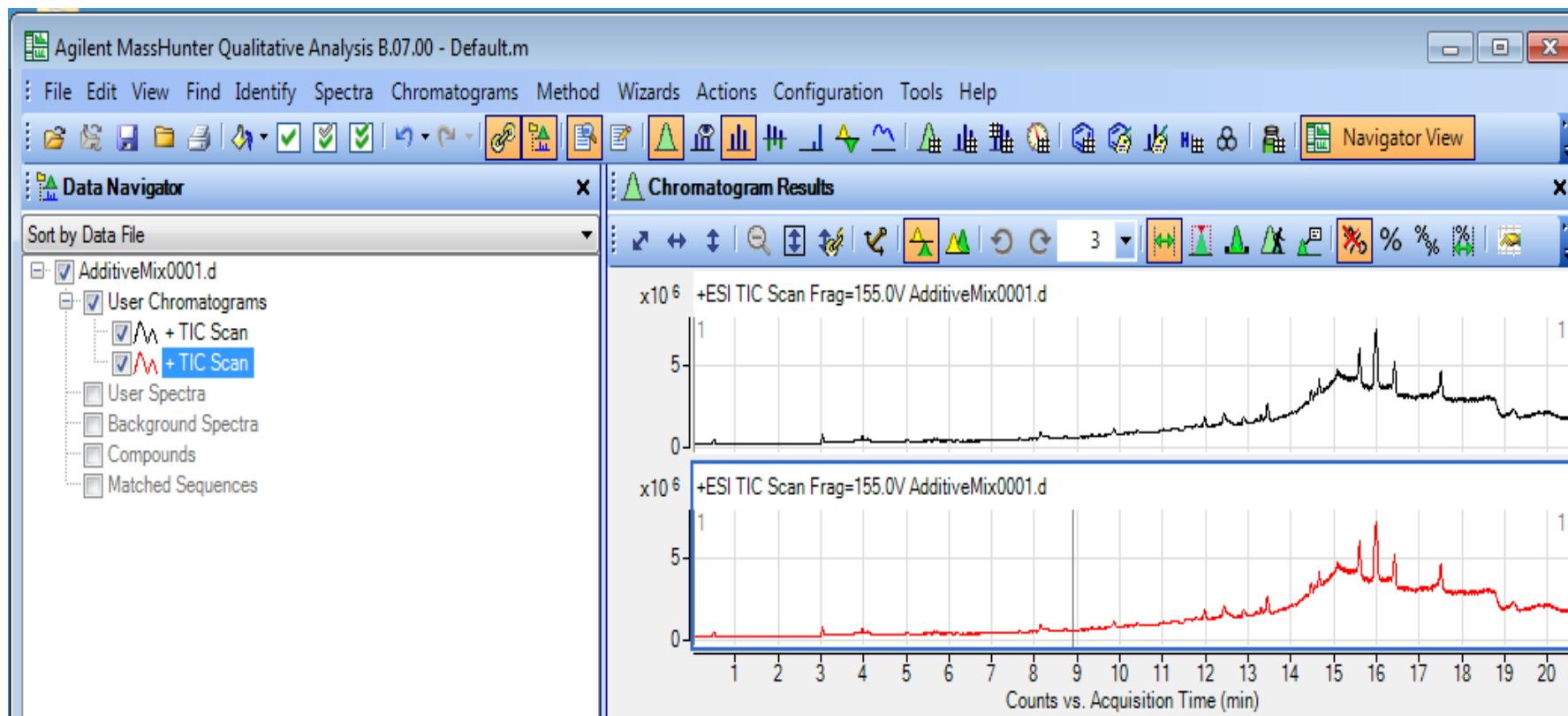
If **Load result data** is checked, the qualitative data manipulations previously saved will be loaded.

If **Run 'File Open' actions from selected method** is checked, automated processing is performed.

If neither **Load result data** or **Run 'File Open' actions from selected method** is checked, then a TIC is automatically extracted from the data files.

# Tip

Every time a data file is loaded see 2 TICs.



# Refresh Data File

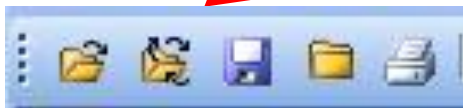
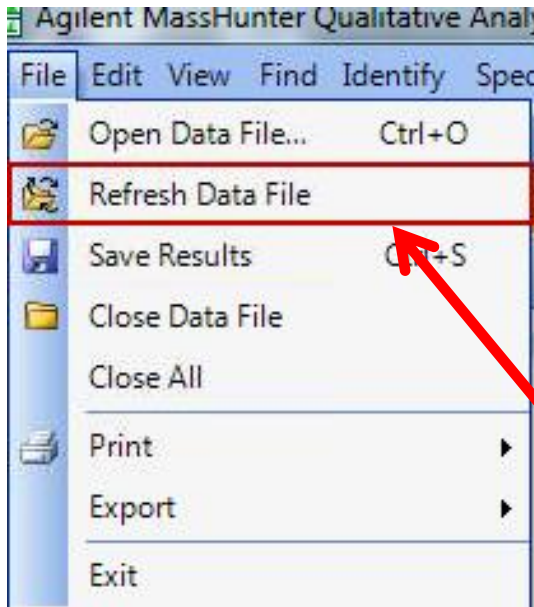
Feature is useful when it is desired to view data as the data file is being acquired.

Initially use **Open Data File** as normal to view data file being acquired.

Then use **Refresh Data File** to update the view and add the most recently acquired data.

**Refresh Data File** is only active if the file is being acquired.

Similar application use for the GC/MSD ChemStation, where it is called SnapShot.





Let's take a moment  
for questions on  
Configuration and  
Layouts.

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Up Next:  
Qualitative Methods

# Qualitative Analysis Methods

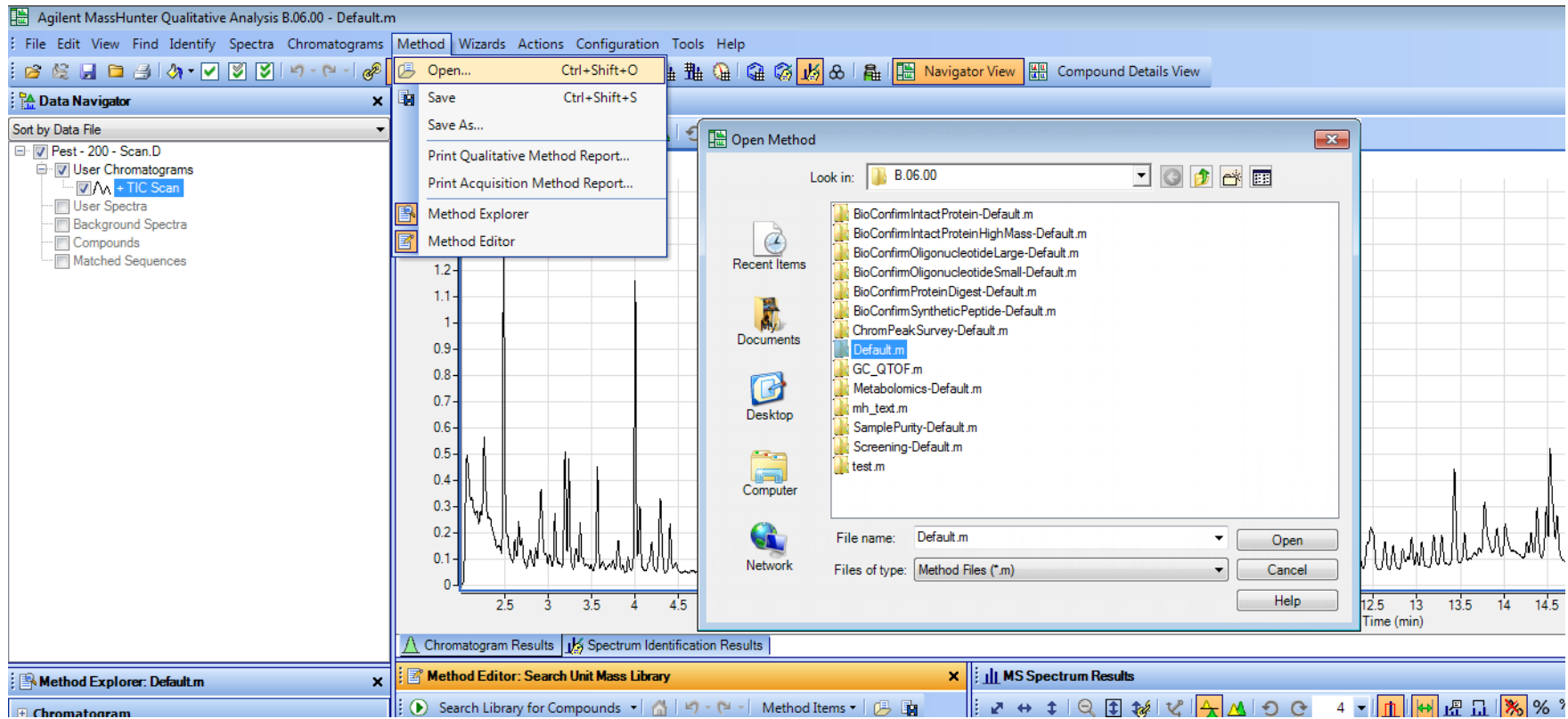
The screenshot displays the Agilent MassHunter Qualitative Analysis B.06.00 interface. The main window shows a chromatogram with a y-axis from 0 to 1.2 and an x-axis from 2.5 to 4.5 minutes. A menu is open, showing options like 'Open...', 'Save', and 'Print Qualitative Method Report...'. An 'Open Method' dialog box is also open, showing a list of method files in the 'B.06.00' folder, with 'Default.m' selected. The 'File name' field is set to 'Default.m' and the 'Files of type' is 'Method Files (\*.m)'. The bottom status bar shows 'Chromatogram Results' and 'Spectrum Identification Results'.

Qualitative Analysis Methods are stored in a .M folder.

Many application & instrument specific methods, generally use Default.m.

Default.M is read-only, after editing “Save As” to a customized method.

# What is a Method? Unified Method Concept



- Qualitative Analysis Methods are stored in a .M folder.
- Quantitative Analysis Methods are stored in a .M folder.
- Quantitative Analysis Reporting Methods are stored in a .M folder.
- Unified method can now be automated to run from the sequence/worklist.

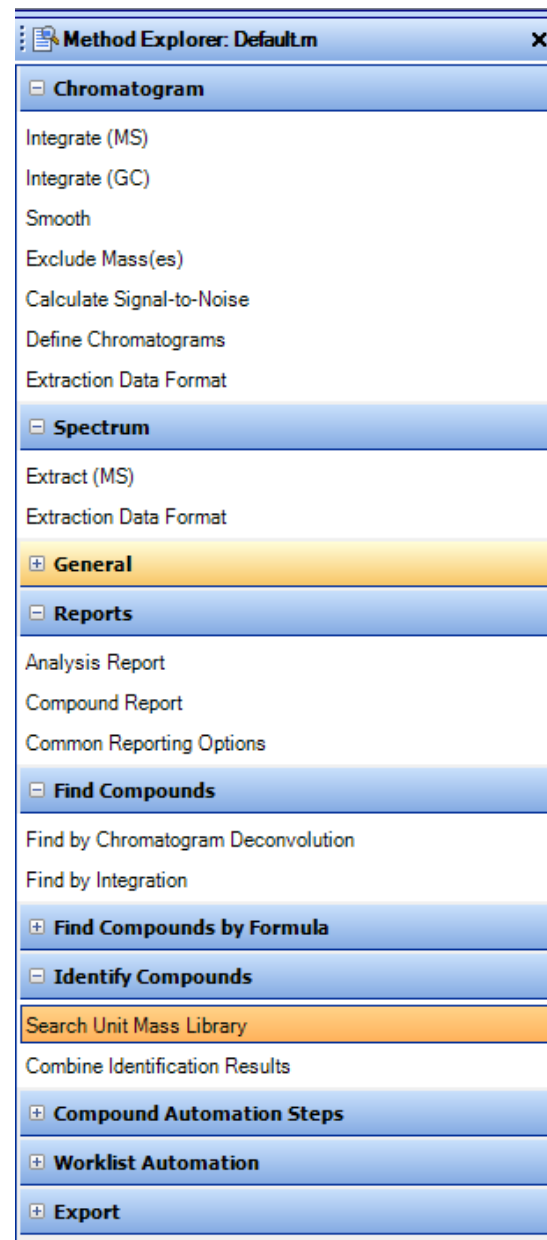


# Method Explorer

Acts as a table of contents for the method.

Items in Method Explorer automatically display related Method Editor features.

Items are dynamic and controlled by the User Configuration and Workflow setting.



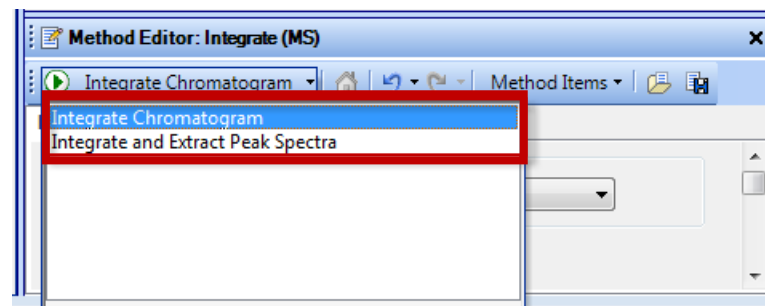
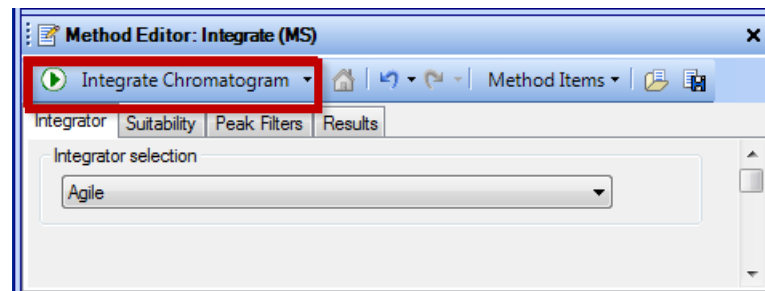
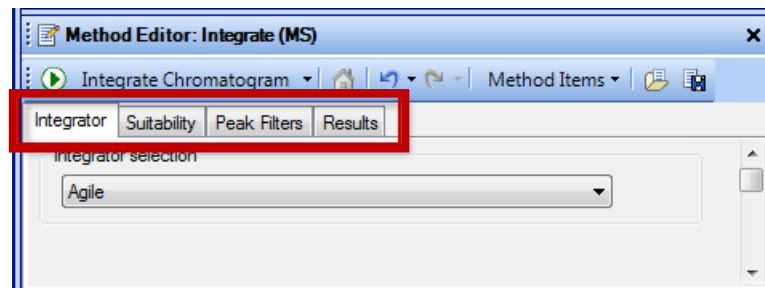
# Method Editor

Display and Edit sections of the Method.

Tabs within the Method Editor further organize method parameters.

The “Run” icon executes the function associated with this part of the method.

In some cases the “Run” icon can have different actions, a drop down list will then display them for selection.



# Relationship Between Action and Method Editor

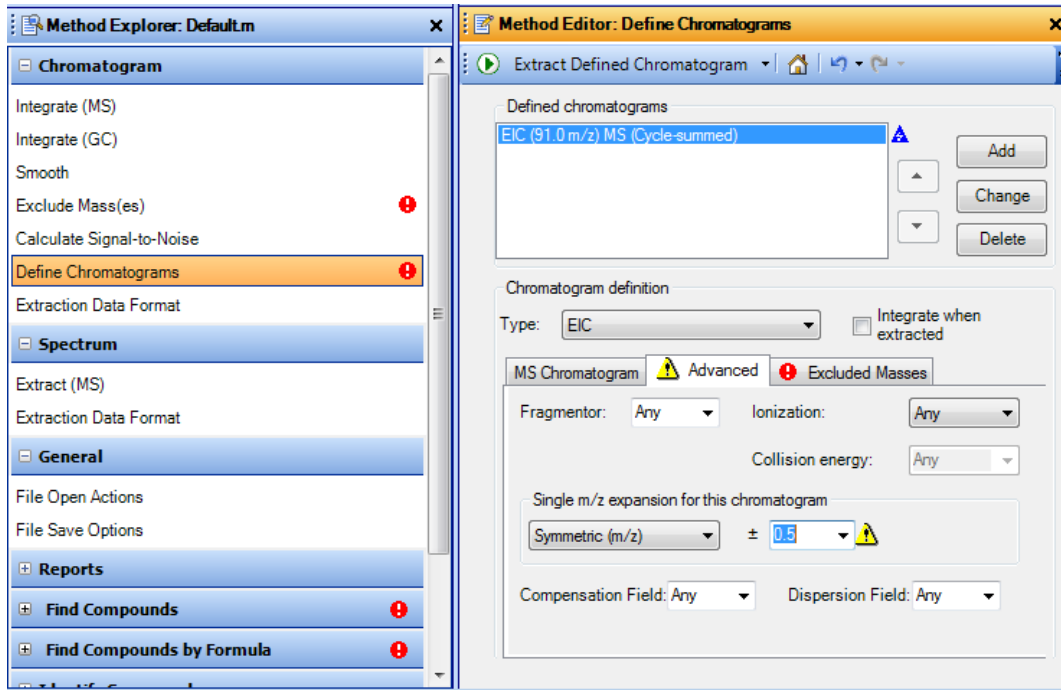
The screenshot displays the Agilent MassHunter Qualitative Analysis B.06.00 - Default.m interface. The **Main Menu** (top) includes options like 'Integrate Chromatogram' and 'Integrate and Extract Peak Spectra'. The **Method Editor: Integrate (MS)** (bottom) shows the configuration for the 'Integrate Chromatogram' action, with a **Run Button** (play icon) to execute the method. The **Right Click Menu** (middle) is shown over a chromatogram plot, mirroring the options in the Main Menu. The chromatogram shows peaks at retention times 8.947, 9.772, and 188.2. The mass spectrum below shows peaks at m/z 114.1, 126.1, 152.1, 162.1, 177.0, and 188.2.

Set parameters for action in Method Editor. Then, perform action.  
Note : The action will be performed on ALL selected (highlighted) items!

# Change and Error Icons



When you make a change to the current method the change is marked. In addition, all other functions that are affected by this change will be marked. Save the **method to remove the icon.**



An invalid value has been entered into a field. The field will reset to the last valid value it contained.



Additional information is required. The error must be fixed before the algorithm will execute.

# Working with Chromatograms

- The power of Qualitative analysis is that you can have more than 1 data file open at a time.
- Extract Chromatograms from Data Files.
- Displaying Chromatograms
  - Selecting for display
  - Zooming
  - Scaling
  - Overlay / List mode
  - Anchoring

# Define Chromatograms

The screenshot displays the Agilent MassHunter Qualitative Analysis B.06.00 - Default.m interface. The main window is divided into several panes:

- Data Navigator:** Shows a tree view of data files including pest-0.05ppm.D, pest-0.1ppm.D, pest-0.2ppm.D, and pest-10ppm.D. Each file has sub-entries for User Chromatograms, User Spectra, Background Spectra, Compounds, and Matched Sequences.
- Chromatogram Results:** Displays four stacked Total Ion Chromatograms (TIC) for the different concentration levels. The x-axis is labeled 'Counts (%) vs. Acquisition Time (min)' and ranges from 4 to 10.5 minutes. The y-axis is labeled '1' and '0'. The traces are color-coded: black (0.05 ppm), red (0.1 ppm), green (0.2 ppm), and blue (10 ppm).
- Method Explorer: Default.m:** Shows a list of methods with 'Define Chromatograms' selected.
- Method Editor: Define Chromatograms:** Shows the configuration for a defined chromatogram. The 'Defined chromatograms' list contains 'BPC (all) MS (Cycle-summed)'. The 'Chromatogram definition' section shows 'Type: BPC' and 'Integrate when extracted' checked. There are 'Add', 'Change', and 'Delete' buttons.
- MS Spectrum Results:** Shows a list of mass spectra results.

A text box in the bottom right corner of the Method Editor pane states: **Can Display:**  
TOF Data  
QTOF Data  
QQQ Data  
SQ Data  
UV data  
FID Data

Data Loaded & Displayed

# Extract Chromatogram

Agilent MassHunter Qualitative Analysis B.06.00 - Default.m

File Edit View Find Identify Spectra Chromatograms Method Wizards Actions Configuration Tools Help

Data Navigator

Sort by Data File

Chromatogram Results

+ TIC Scan pest-0.1ppm.D

TIC Scan pest-0.2ppm.D

TIC Scan pest-10ppm.D

Extract Chromatograms

List of opened data files

- pest-0.05ppm.D
- pest-0.1ppm.D
- pest-0.2ppm.D
- pest-10ppm.D

Type: EIC  Integrate when extracted

MS Chromatogram Advanced Excluded Masses

MS level: All Polarity: Positive

Scans: All scan types

m/z of interest: Any

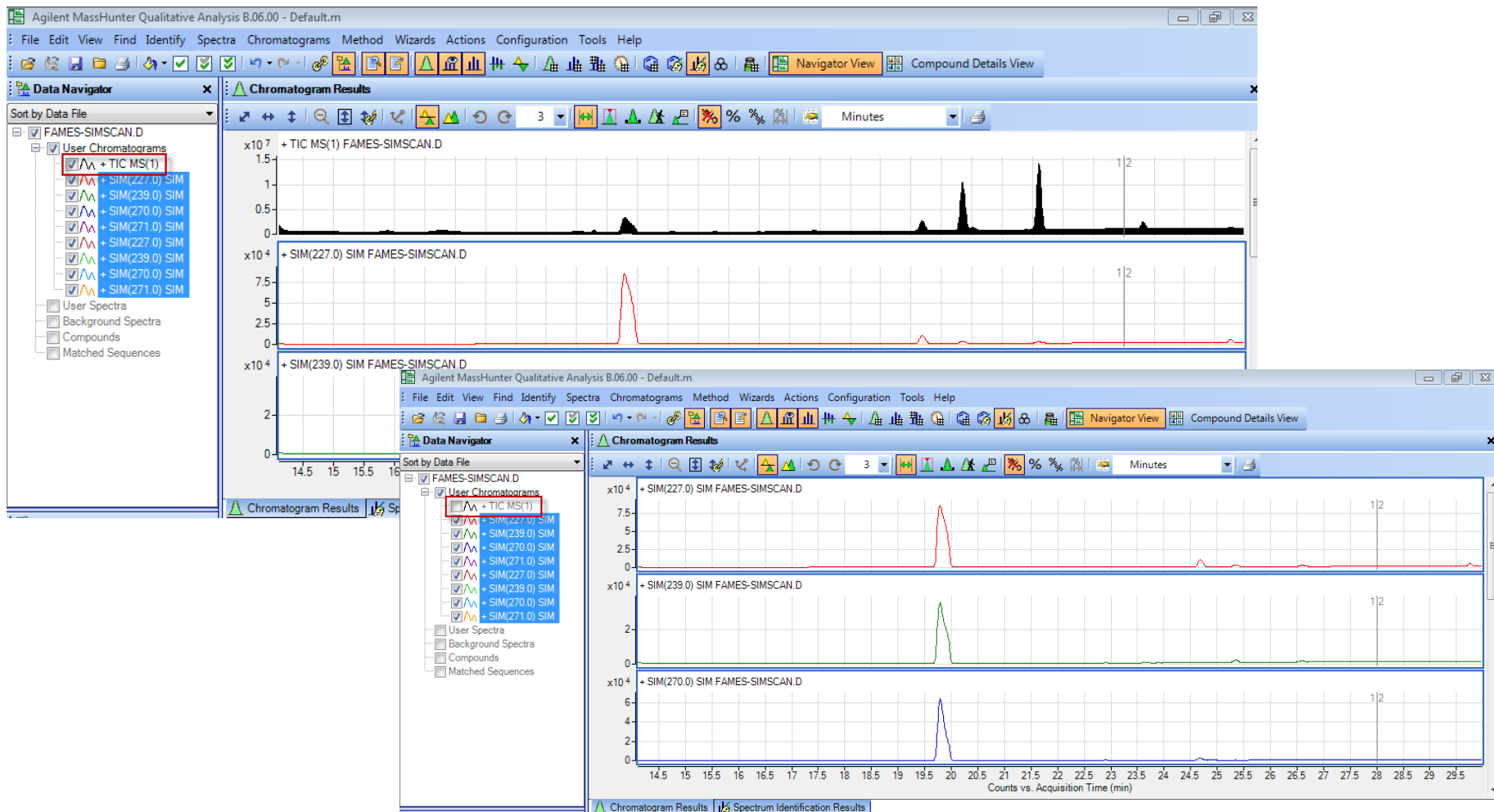
m/z value(s): 91.1

Merge multiple masses into one chromatogram

OK Cancel

List of Chromatogram types is determined by data in file.

# Selecting Chromatograms for Display

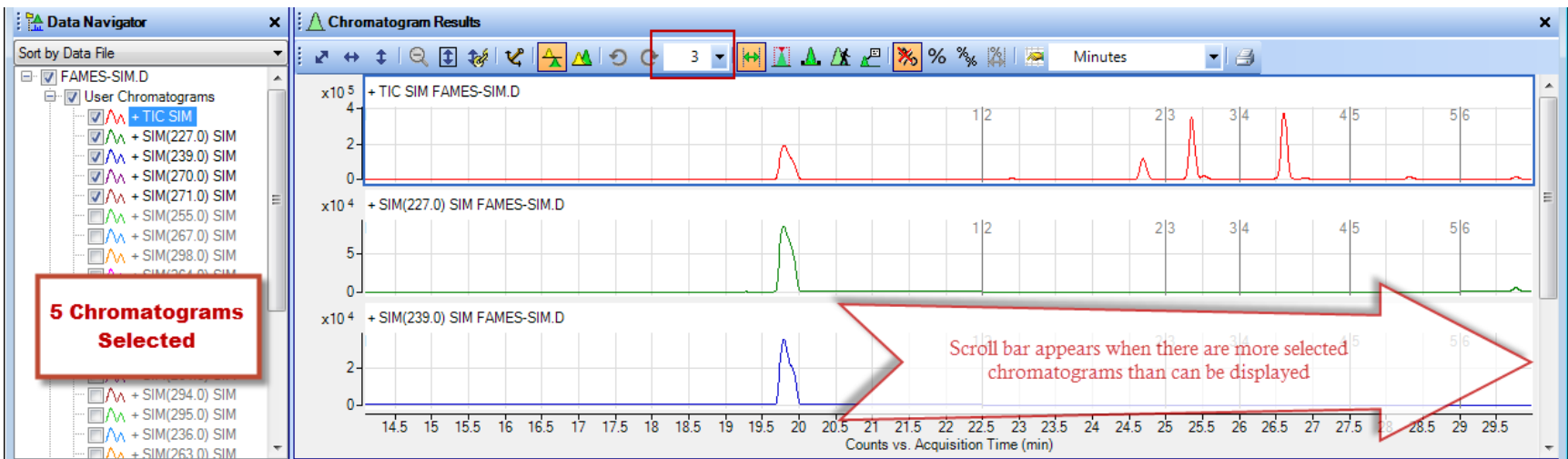
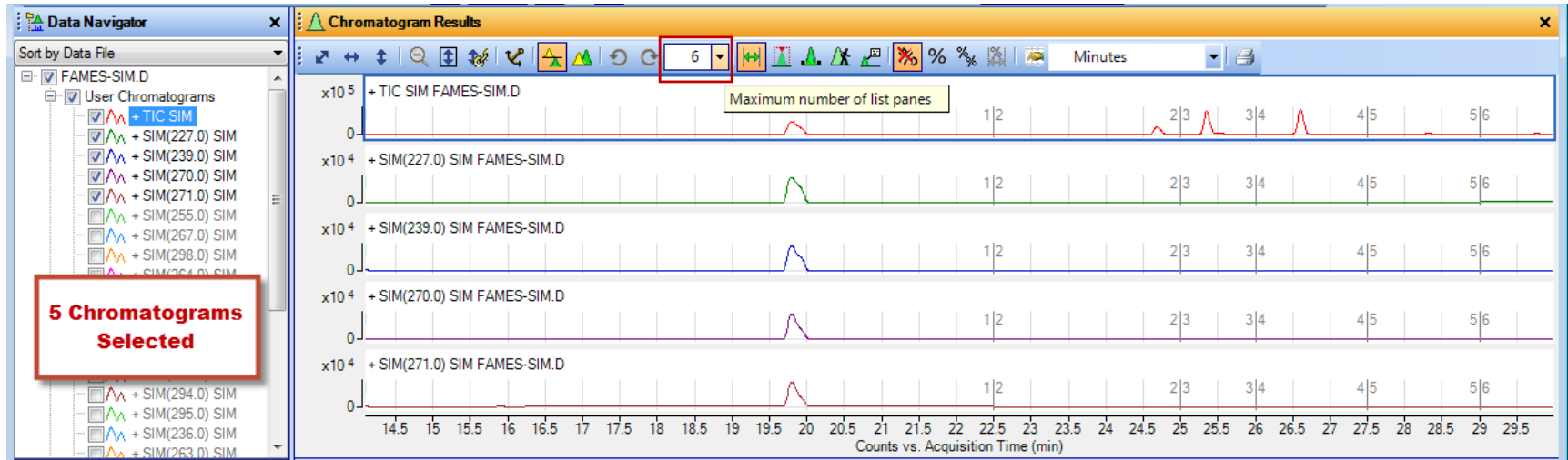


Items in the Data Navigator, like Chromatograms, will be displayed if checked and not displayed if unchecked.



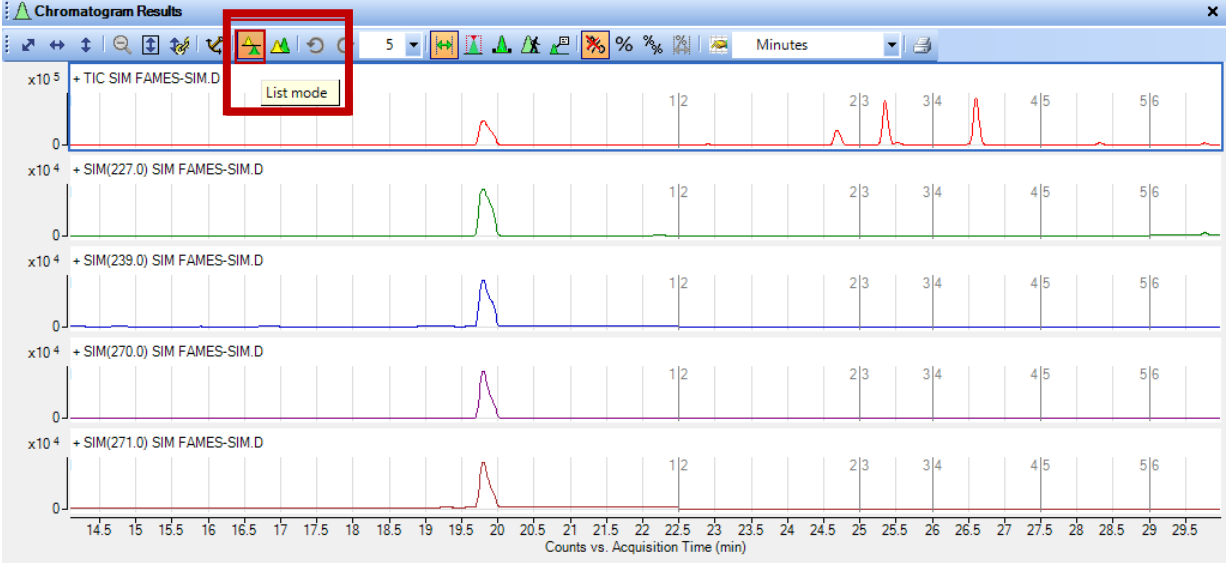
# Specify Number of Chromatograms Displayed

Maximum number of chromatograms to display in window, may be fewer.

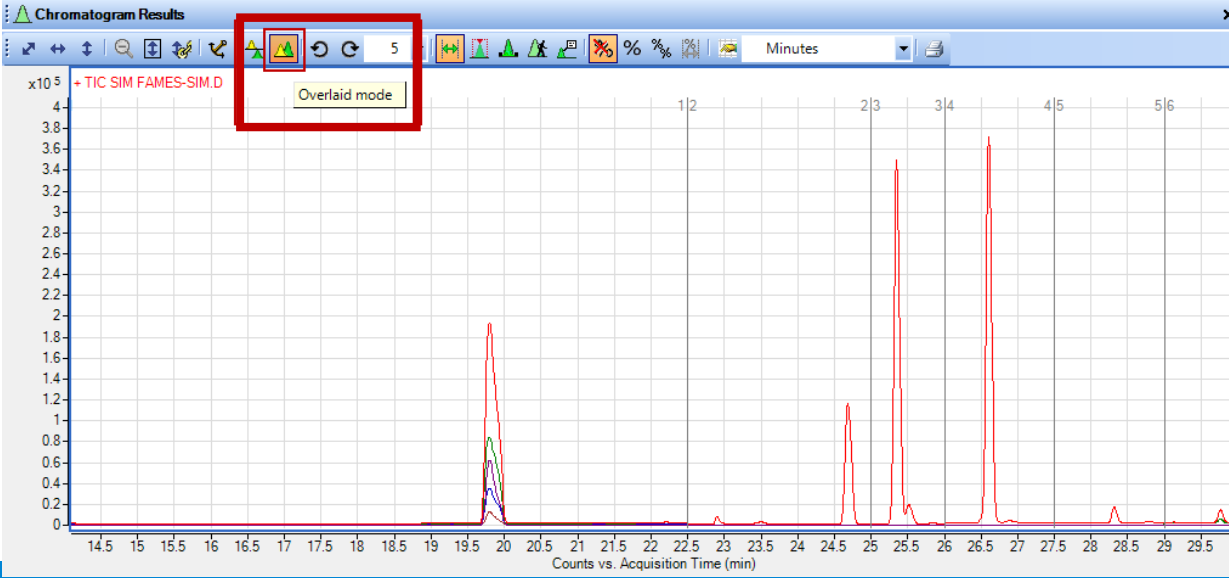
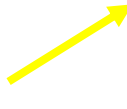


# Overlay vs. List Mode Chromatograms

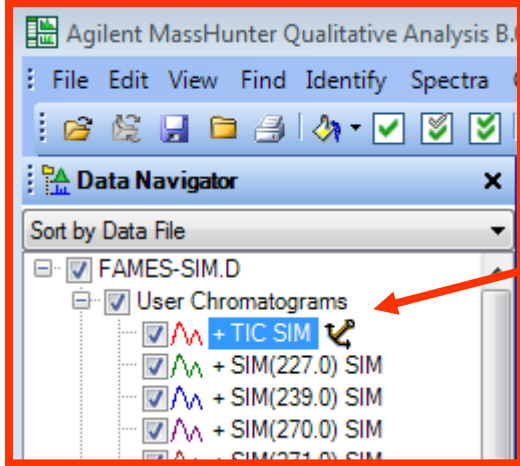
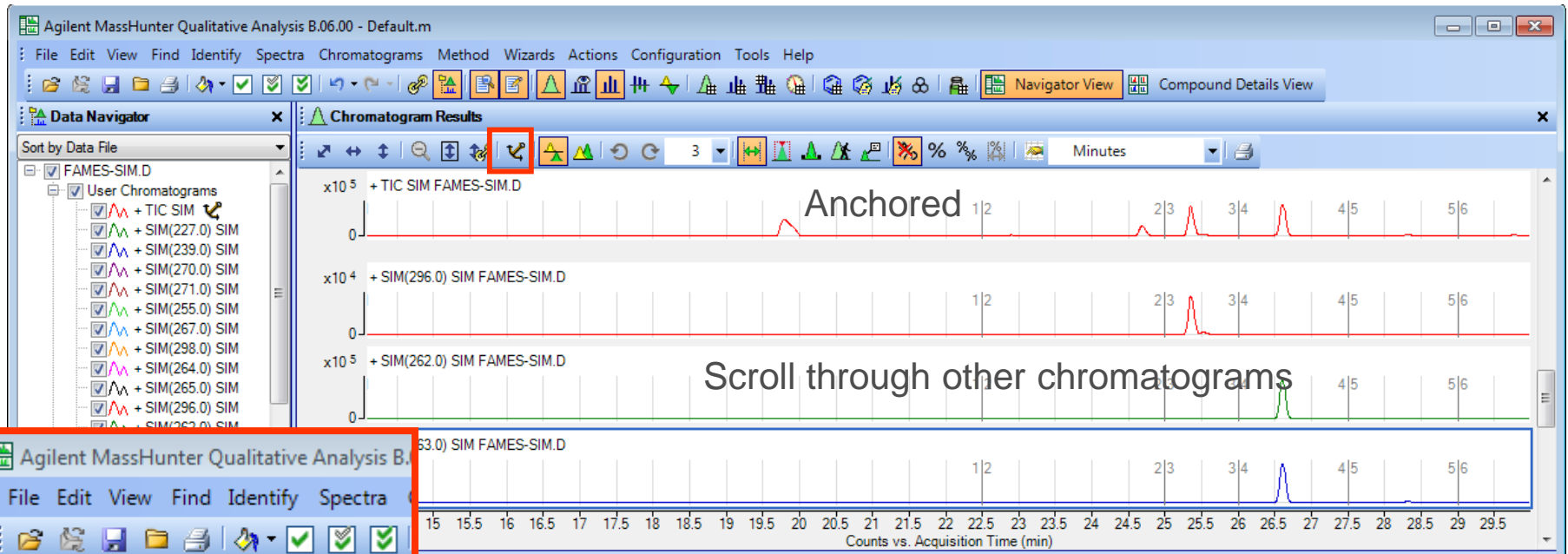
List  
(Separated)



Overlaid



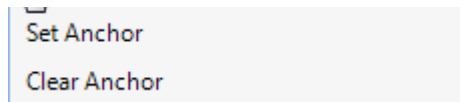
# Anchoring



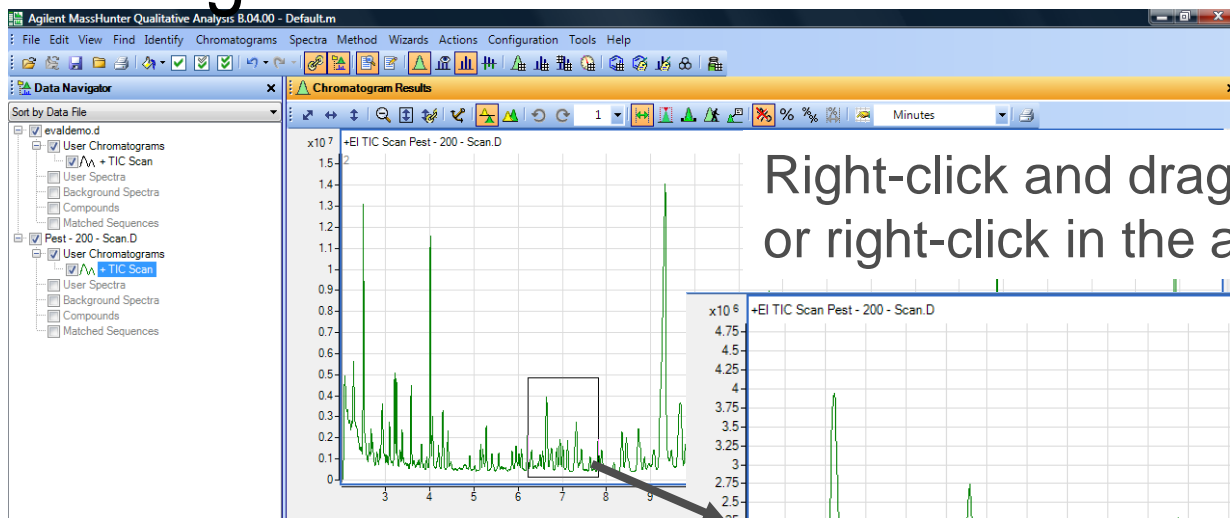
Setting an anchor keeps the anchored chromatogram displayed at all times.

Only one Chromatogram can be anchored at a time.

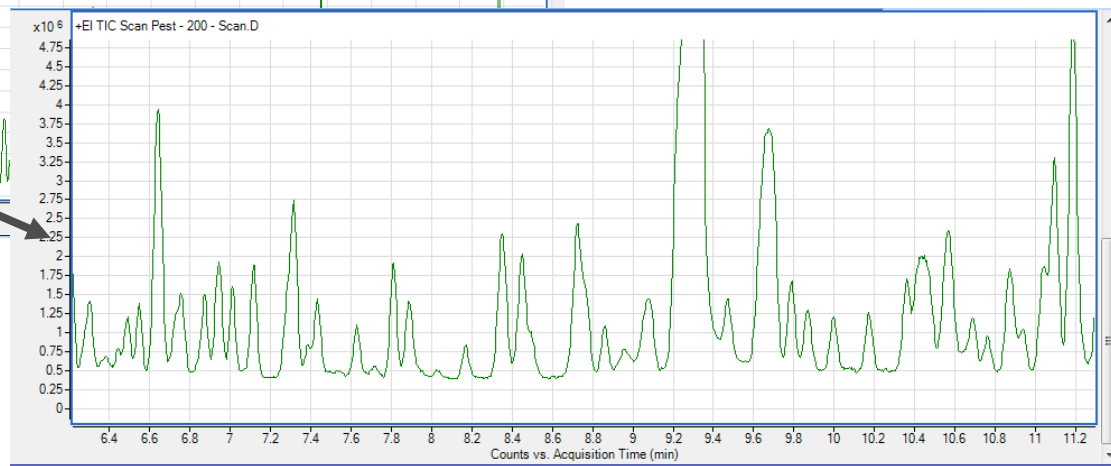
The anchor can be set and cleared from the context menu.



# Zooming



Right-click and drag over the desired area or right-click in the axis and drag to zoom.



**Autoscale**



**Unzoom**

**(multiple levels)**

**Linked Y-axis**

**Normalization**



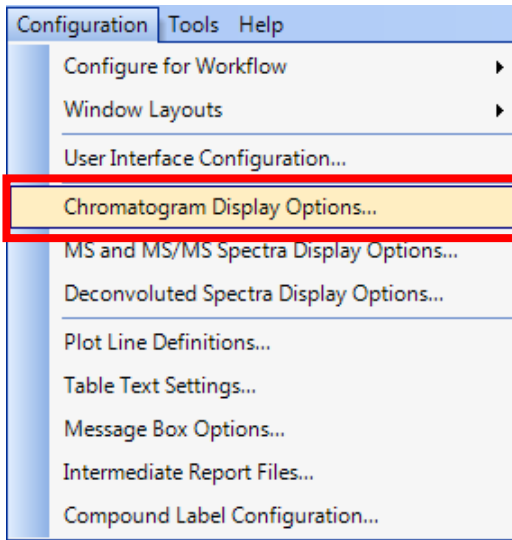
Scale to largest in Each over Selected Range

Scale to largest in Each

Scale to largest in All

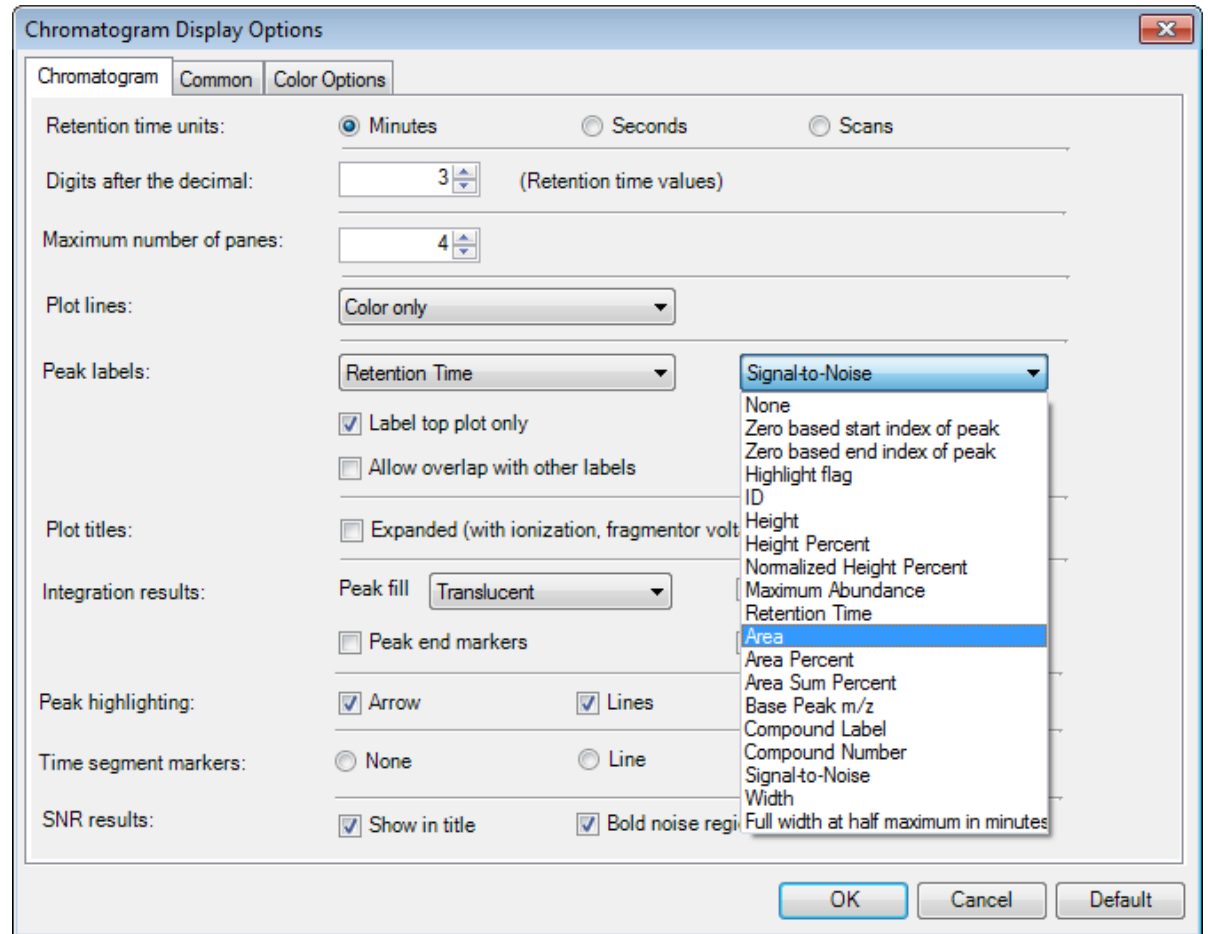
Scale Off

# Chromatogram Display Options



Main Menu

Within Display



Customize Appearance of Chromatograms



Let's take a moment  
for questions on  
Chromatogram Display  
Options.

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Up Next:  
Chromatogram Functions

# Chromatogram Functions

## Chromatogram

Integrate (MS)

Integrate (GC)

Smooth

Exclude Mass(es)

Calculate Signal-to-Noise

Define Chromatograms

Extraction Data Format

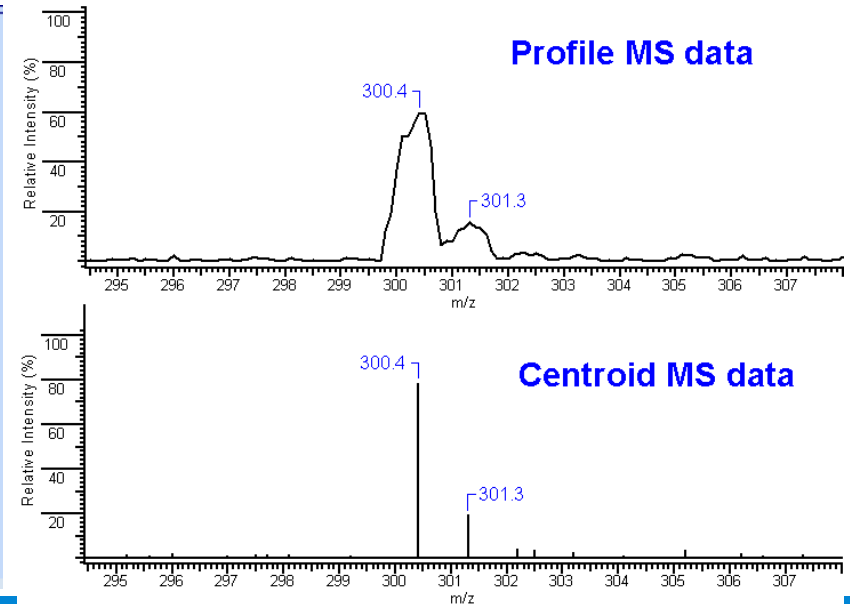
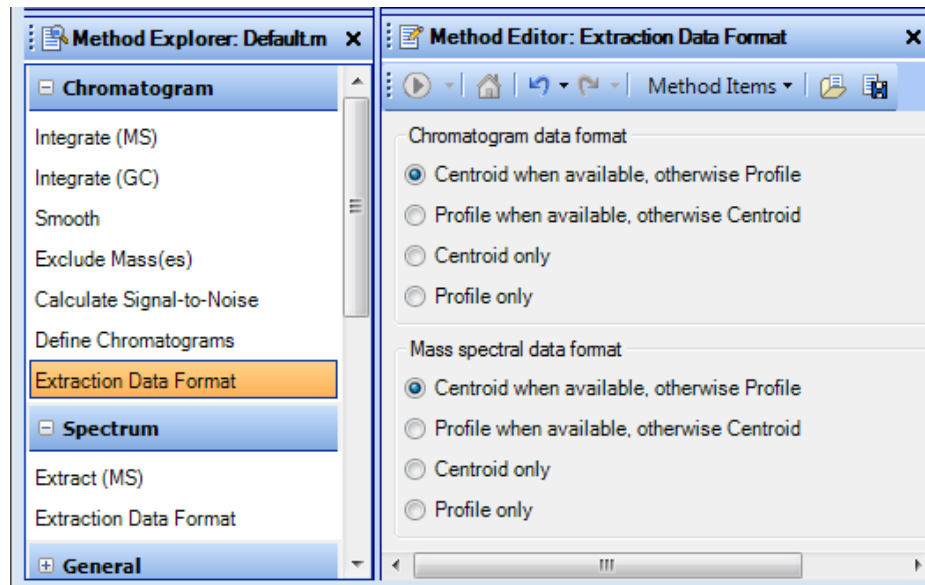
Identify chromatographic peaks for further analysis.

Exclude certain masses when depicting chromatograms.

Extract chromatograms.

# Extraction Data Format - Profile and Centroid

- Data files may contain Centroid, Profile (Raw) or both data types.
- Settings determine which type is used to create chromatograms / spectra.
- Centroid data is the most commonly used, ~10 times smaller than Profile
- Profile is useful for mass peak area comparisons such as when optimizing acquisition parameters, i.e. finding the mass defect or center of mass centroid
- How is Profile Data activated?





# Extract Defined Chromatogram

The screenshot displays the Agilent MassHunter Qualitative Analysis 8.06.00 interface. The 'Data Navigator' on the left shows a tree view of data files. The 'Method Editor' in the center shows the 'Define Chromatograms' section, where 'BPC (all) MS (Cycle-summed)' is selected. The 'Extract Defined Chromatograms' dialog box is open, showing a list of opened data files: pest-0.05ppm.D, pest-0.1ppm.D, pest-0.2ppm.D, and pest-10ppm.D. The 'Chromatogram definition' section shows 'Type: BPC' and 'Integrate when extracted' checked. The 'MS Chromatogram' tab is active, showing 'Fragmentor: Any', 'Ionization: Any', and 'Collision energy: Any'. The 'Single m/z expansion for this chromatogram' is set to 'Symmetric (m/z) ± 0.5'. The 'Extract' button is highlighted.

Software extracts a list of chromatograms which are stored in the Extract Defined Chromatogram section of the method.

List of Chromatogram types is fixed list of all instrument types.

# Extract Define Chromatograms

- Select MS Level based on acquisition scan type.

The screenshot displays the Agilent MassHunter Qualitative Analysis software interface. The top panel shows the 'Chromatogram Results' window with a Total Ion Chromatogram (TIC) plot. The y-axis is labeled 'x10<sup>6</sup> +EI TIC Scan Pest - 200 - Scan.D' and ranges from 0 to 4.75. The x-axis represents time in minutes, ranging from 6.4 to 8.0. The plot shows several peaks, with the most prominent one at approximately 6.6 minutes. The bottom panel shows the 'Method Editor: Define Chromatograms' dialog box. The 'Defined chromatograms' list contains 'BPC (all) MS (Cycle-summed)'. The 'Chromatogram definition' section shows 'Type: BPC' and 'Integrate when extracted' checked. The 'MS Chromatogram' section is expanded, showing 'MS level: MS', 'Polarity: Both', 'Scans: All single stage scan types', 'm/z of interest: Any', and 'm/z value(s):'. A green arrow points to the 'Change' button in the 'Defined chromatograms' list.

## Types of Chromatograms

**TIC** – Total Ion Chromatogram

**BPC** – Base Peak Chromatogram

**EIC** – Extracted Ion Chromatogram

**SIM** – Selected Ion Monitor

**Other Chromatograms** – GC, DAD, ADC

**Instrument Curve (LC)** - %Comp., Temps, etc.

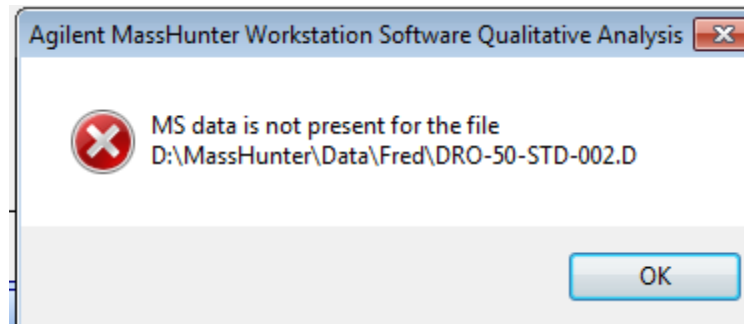
**Triple Quad** systems only

**MRM** – Multiple Reaction Monitor

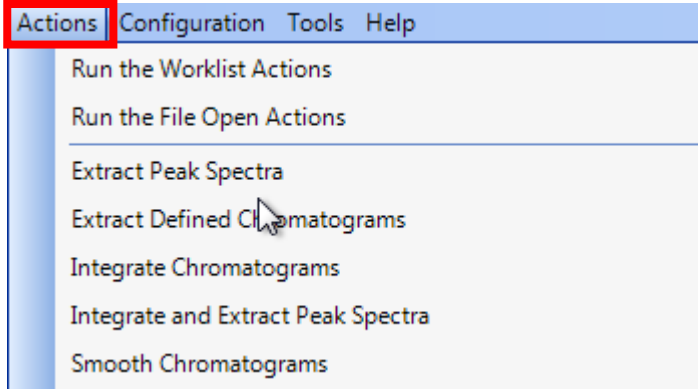
**pNLC** - Precursor Neutral Loss Chromatogram

**Tip: Select Change or Add.**

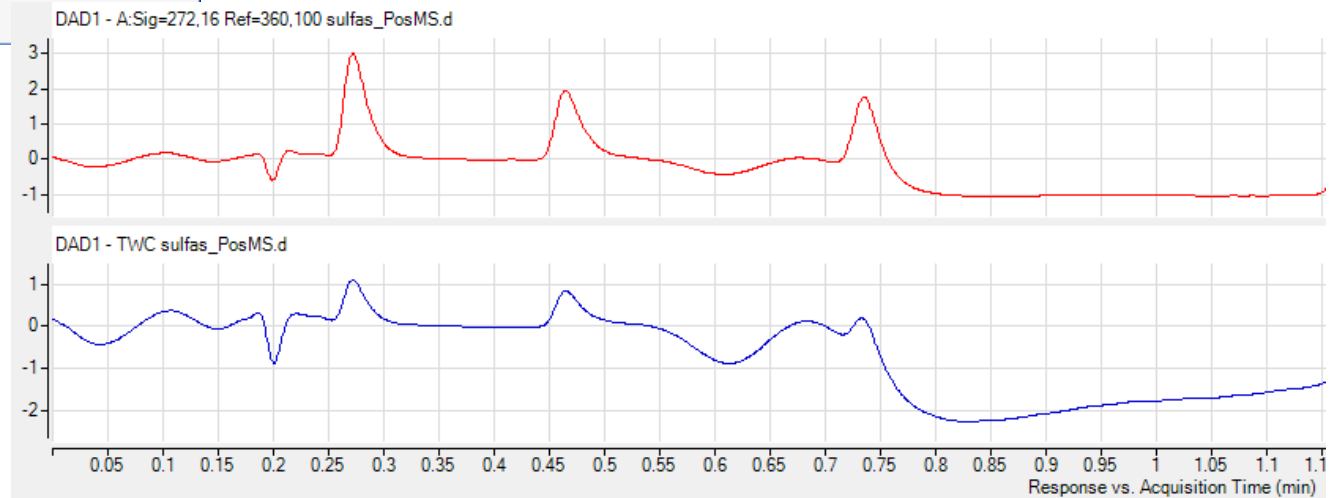
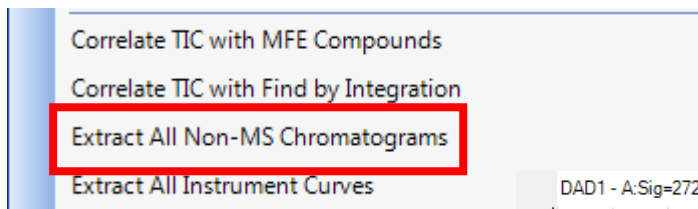
# Extracting GC, UV and other Non-MS Signals



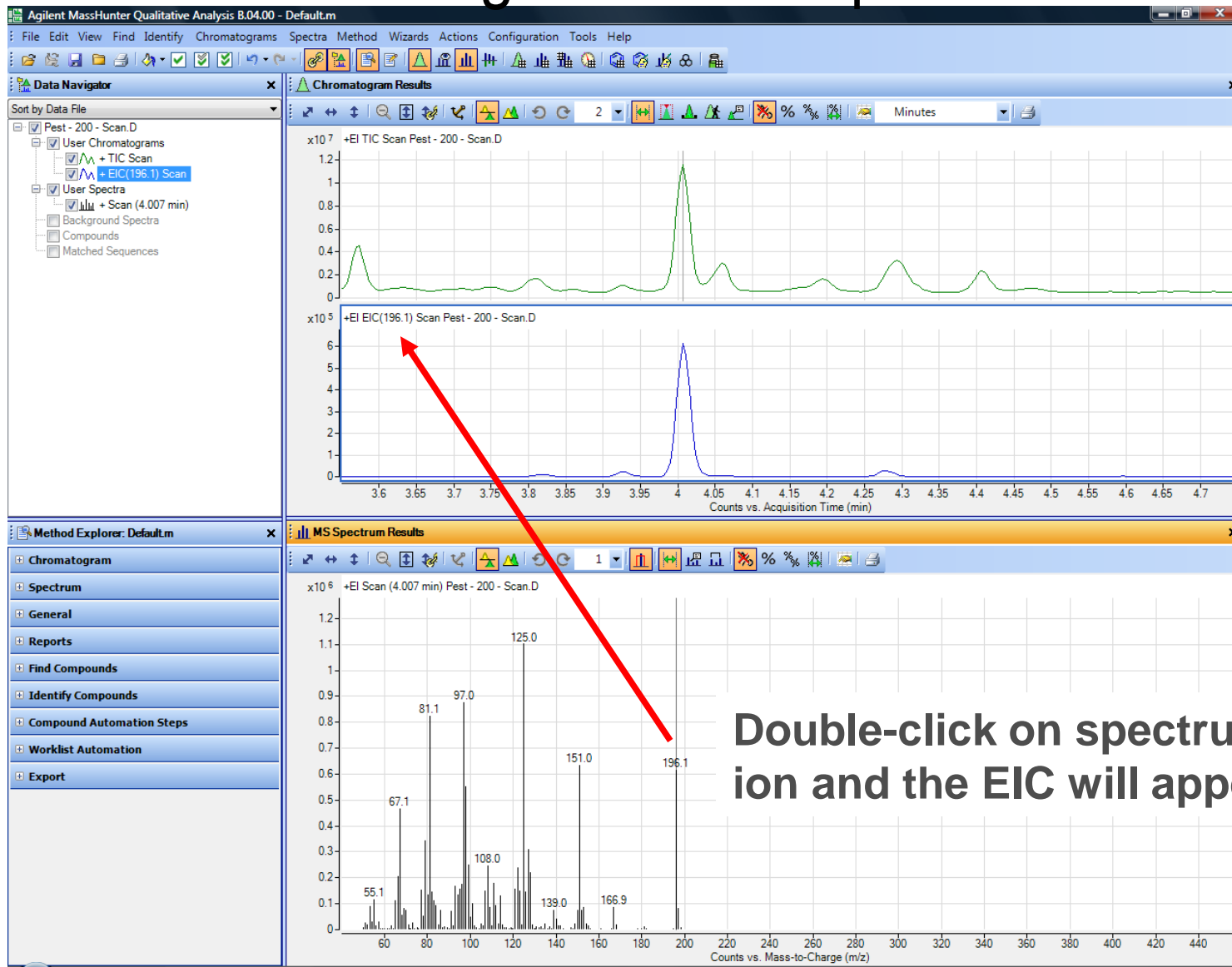
# Extract All Non-MS Chromatograms



Select **Actions > Extract All Non-MS Chromatograms.**



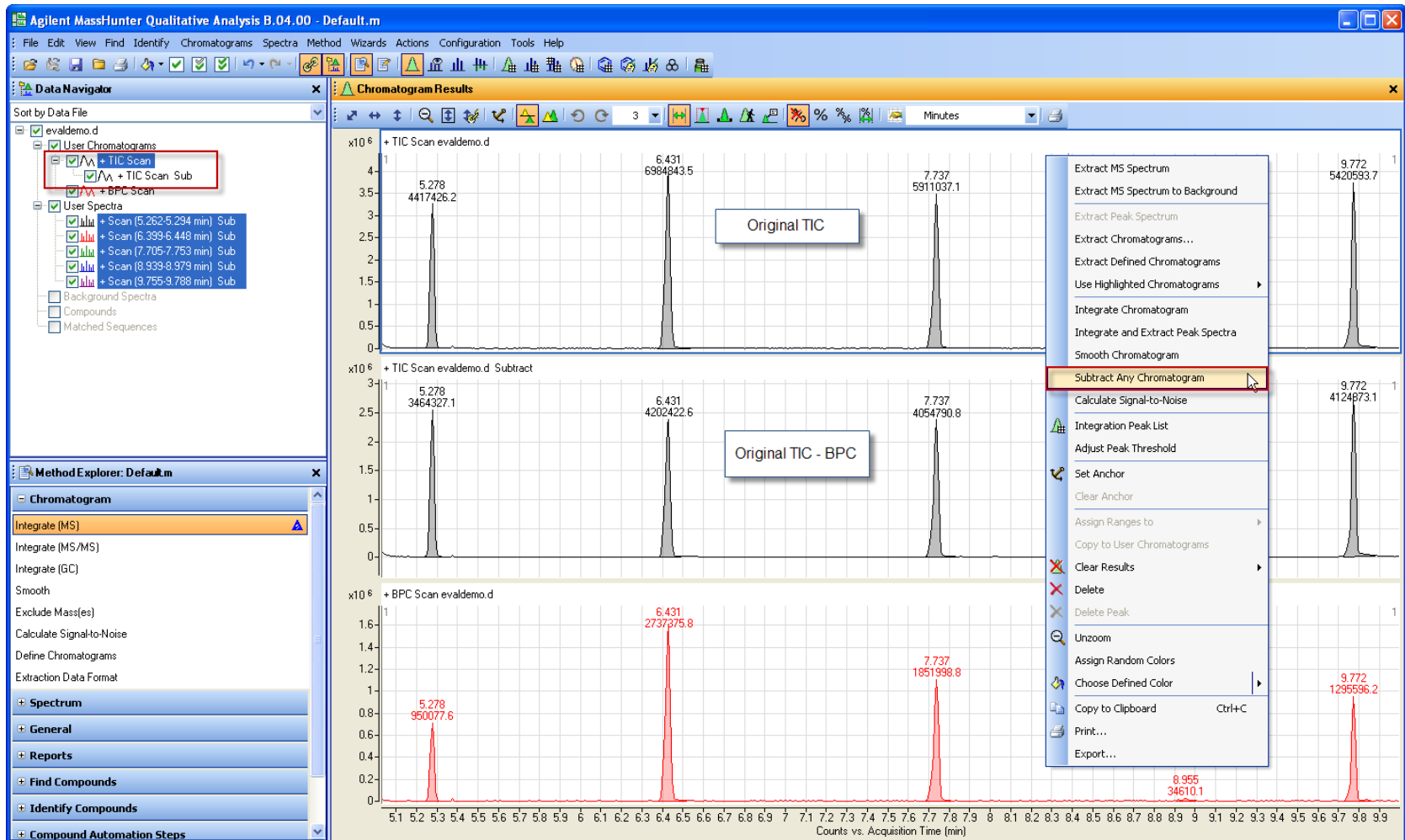
# Extract Ion Chromatograms from Spectra



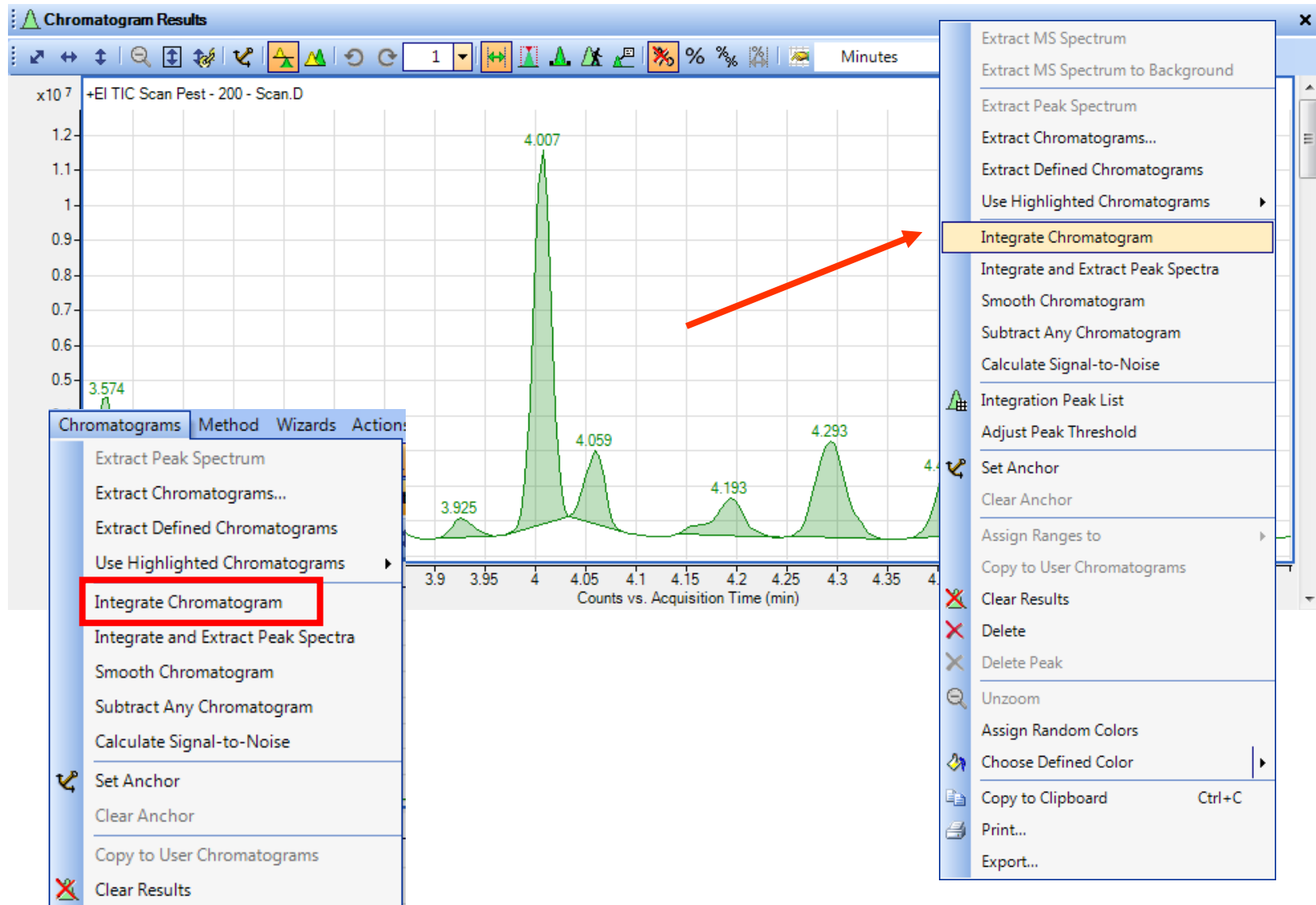
**Double-click on spectrum ion and the EIC will appear.**

# Subtract Any Chromatogram

Right click in Chromatogram, select “Subtract Any Chromatogram”, the next chromatogram you click on will be subtracted from 1<sup>st</sup> one.



# Integrate Chromatogram



# Integrate Chromatogram

Independent Integrator for each configuration.

The screenshot displays the Agilent software interface for integrating a chromatogram. On the left, the 'Method Explorer' shows a tree view with 'Chromatogram' expanded, and 'Integrate (MS)' selected. Below it are other methods like 'Integrate (MS/MS)', 'Integrate (UV)', 'Integrate (GC)', 'Integrate (ADC)', 'Smooth', 'Exclude Mass(es)', 'Calculate Signal-to-Noise', 'Define Chromatograms', 'Adjust Delay Time', 'Extraction Data Format', 'Spectrum', 'General', 'Reports', and 'Find Compounds'. The main window is titled 'Method Editor: Integrate (MS)' and has three tabs: 'Integrator', 'Suitability', and 'Peak Filters'. The 'Integrator' tab is active, showing 'Integrator selection' with 'Agile 2' selected. Below this are other integrator options: 'ChemStation', 'General', 'Universal', 'MS/MS', 'MS/MS (GC)', 'Agile', and 'Agile 2'. The 'Results' tab is also visible, showing 'Previous results' with a checked 'Clear previous peak spectra' option and 'New results' with 'Highlight first peak' selected. The 'Filter on' section shows 'Peak area' selected, with 'Height filters' and 'Area filters' sections. The 'Area filters' section has 'Relative area' checked and set to 1.000. The 'Maximum number of peaks' section has 'Limit (by height) to the largest' checked and set to 100.



# Integrator Types

## **Agile2**

- Default Integrator, 3<sup>rd</sup> generation parameterless integrator
- Better baselines, higher sensitivity to smaller peaks

## **Agile**

- 2<sup>nd</sup> generation parameterless integrator

## **Universal**

- 1<sup>st</sup> generation ChemStation integrator
- Familiar to ChemStation users

## **General (RTE)**

- Familiar to MSD ChemStation users
- Areas in Universal are 10 time smaller than seen in ChemStation

## **MS/MS and MS/MS (GC)**

- 1<sup>st</sup> generation parameterless integrator intended for MS/MS systems, not recommended for SQ. Originally required 64 data points.

## **ChemStation**

- 2<sup>nd</sup> generation ChemStation
- Intended for UV

# Integration Peak List



The screenshot displays the Agilent MassHunter Qualitative Analysis interface. The main window shows a Total Ion Chromatogram (TIC) with several peaks labeled with their retention times: 1.092, 1.729, 2.801, 4.387, 5.862, 7.794, 9.391, 10.330, 12.050, 12.899, and 14.440. A table titled 'Peaks: + TIC Scan' is open, showing the following data:

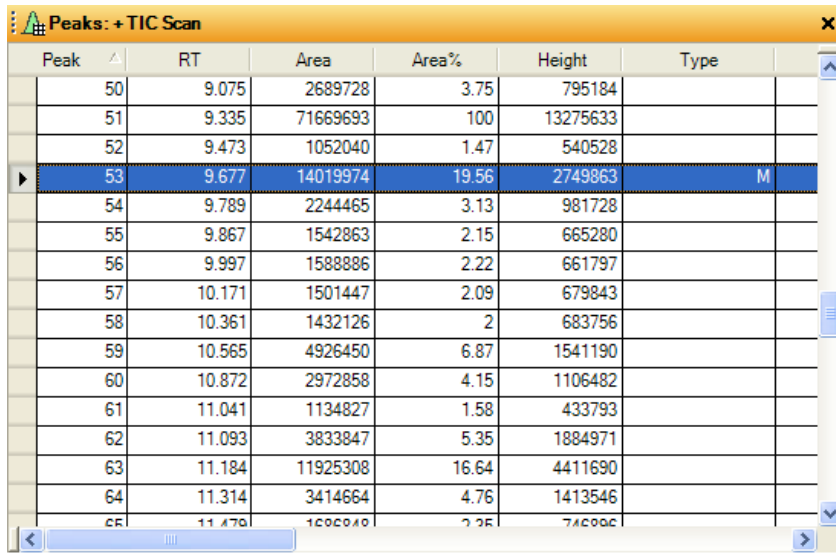
Peak	RT	Area	Height	Type	Width	FWHM	SN
1	1.092	77952859.41	7612918.32		0.413	0.169	
2	1.383	10235578.69	1959827.69		0.112	0.11	
3	1.461	8212983.73	1688114.13		0.134	0.075	
4	1.651	8609082.19	1915739.45		0.112	0.074	
5	1.729	134576403.06	7221652.96		0.447	0.327	
6	2.198	55317944.2	5686861.89		0.503	0.133	
7	2.801	30883808.17	4659114.77		0.257	0.102	
8	3.069	51566225.25	2944294.61		0.503	0.278	
9	3.471	2444509.65	425827.79		0.112	0.123	
10	3.594	23868582.69	2047197.44		0.547	0.154	
11	4.387	37076639.75	4150100.73		0.67	0.14	
12	5.862	18625684.03	1319444.43		0.771	0.235	

An 'Add/Remove Columns' dialog box is open, showing a list of available columns and a list of columns to be displayed. The 'Available Columns' list includes: Area Sum %, Baseline, End, End BL Y, End Y, Height %, Height % (Norm), Start, Start BL Y, and Start Y. The 'Show these columns' list includes: Area, Area %, Base Peak, Cpd, Flags (Tgt), FWHM, Height, k', Label, Max Y, Peak, Plates, Plates/M, Resolution, RT, SNR, Symmetry, Tailing factor, Type, and Width. Buttons for 'Add ->', '<- Remove', 'Add All ->>', '<<- Remove All', 'OK', and 'Cancel' are visible.

Right-click on the Peak List header to Add/Remove Columns.

Tip: Tables can be configured.

# Integration Peak Tables (all tables)



Peak	RT	Area	Area%	Height	Type
50	9.075	2689728	3.75	795184	
51	9.335	71669693	100	13275633	
52	9.473	1052040	1.47	540528	
53	9.677	14019974	19.56	2749863	M
54	9.789	2244465	3.13	981728	
55	9.867	1542863	2.15	665280	
56	9.997	1588886	2.22	661797	
57	10.171	1501447	2.09	679843	
58	10.361	1432126	2	683756	
59	10.565	4926450	6.87	1541190	
60	10.872	2972858	4.15	1106482	
61	11.041	1134827	1.58	433793	
62	11.093	3833847	5.35	1884971	
63	11.184	11925308	16.64	4411690	
64	11.314	3414664	4.76	1413546	
65	11.479	1600048	2.25	740006	

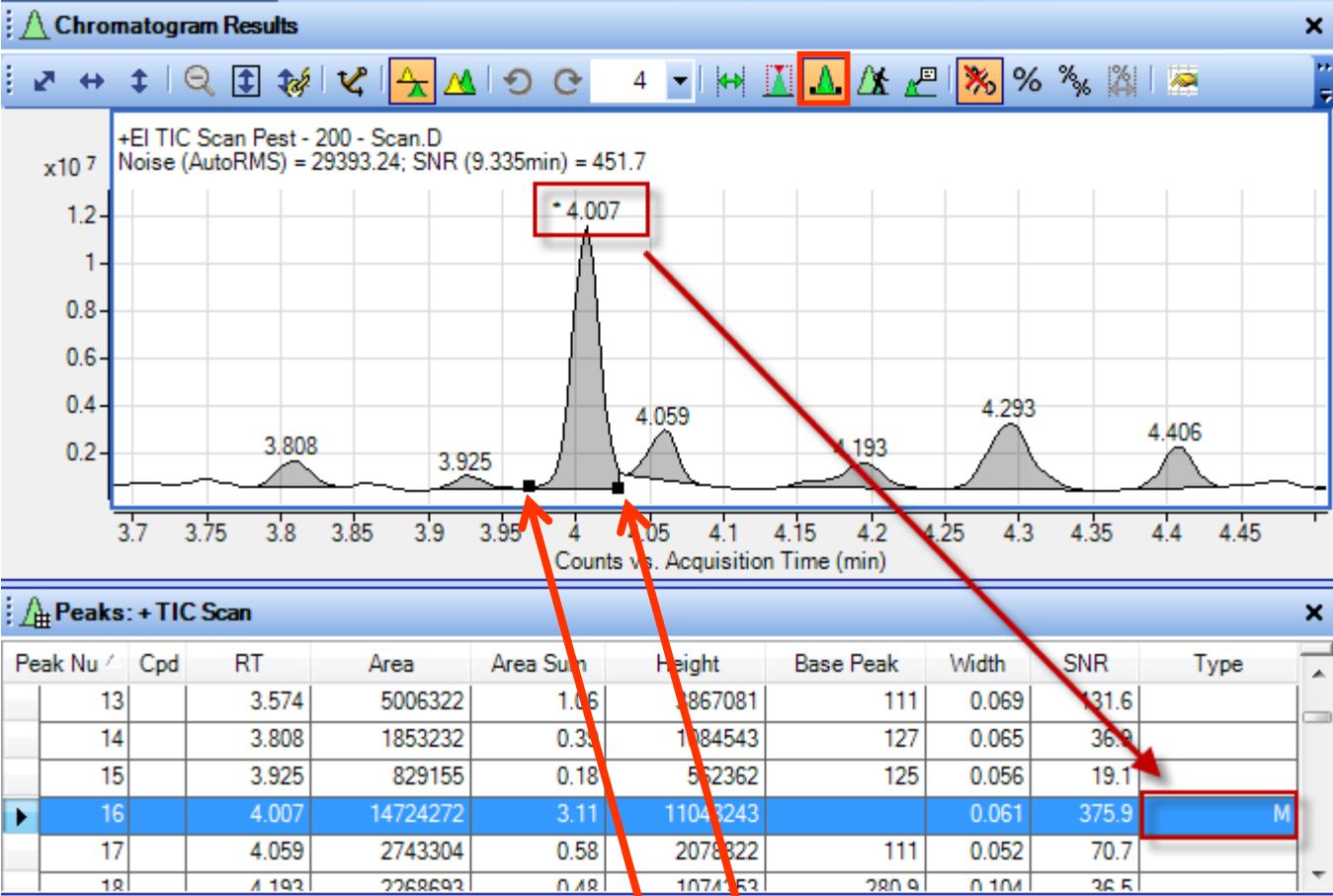
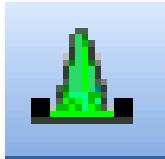
Tables can be moved to different locations.

Tables can be split for easy viewing.

Columns can be added, removed, and moved.

Columns can be moved by Clicking and dragging on column header.

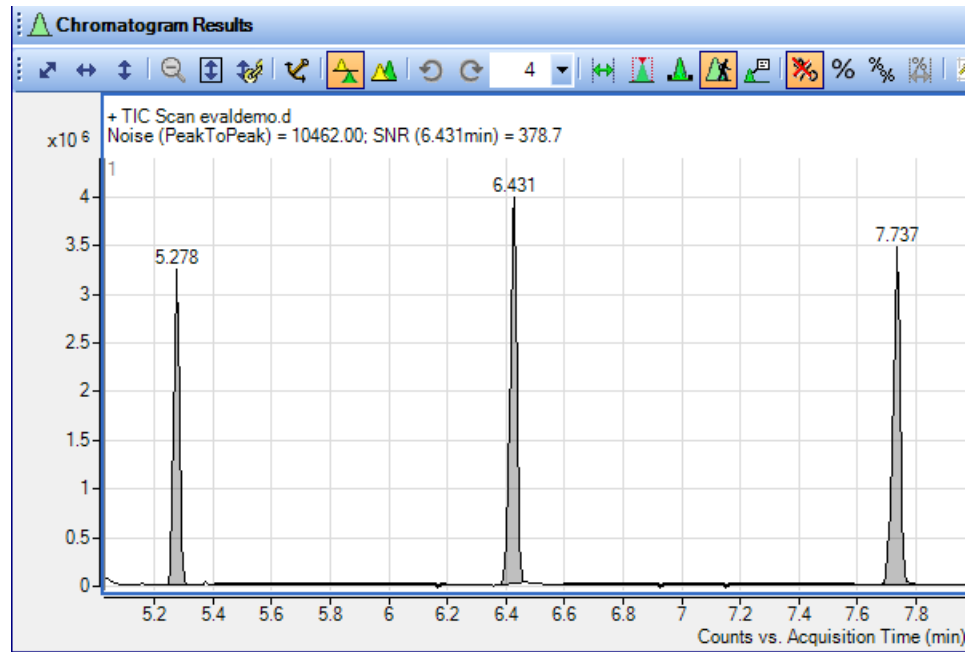
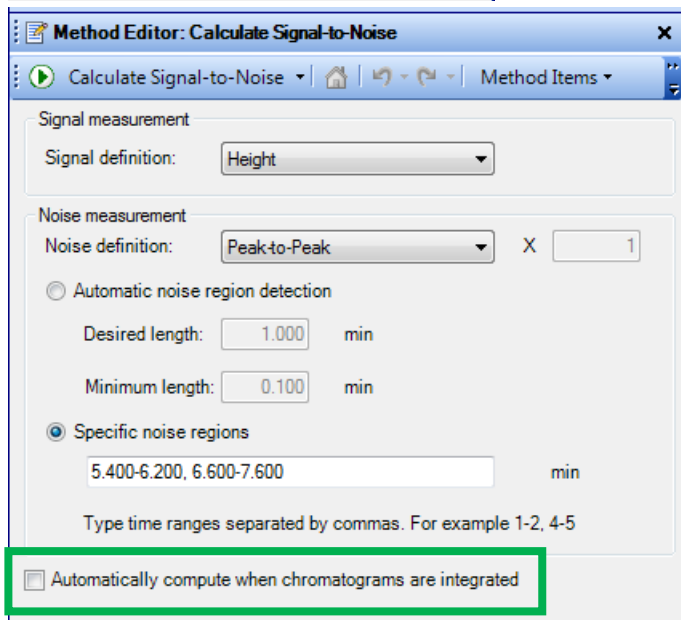
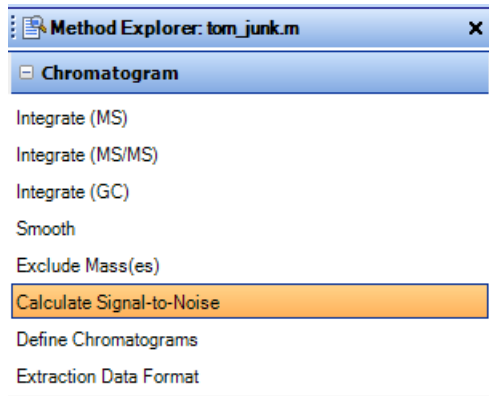
# Manual Integration



Use mouse cursor to manually integrate peak.

# Calculate Signal-to-Noise Specific Noise Regions

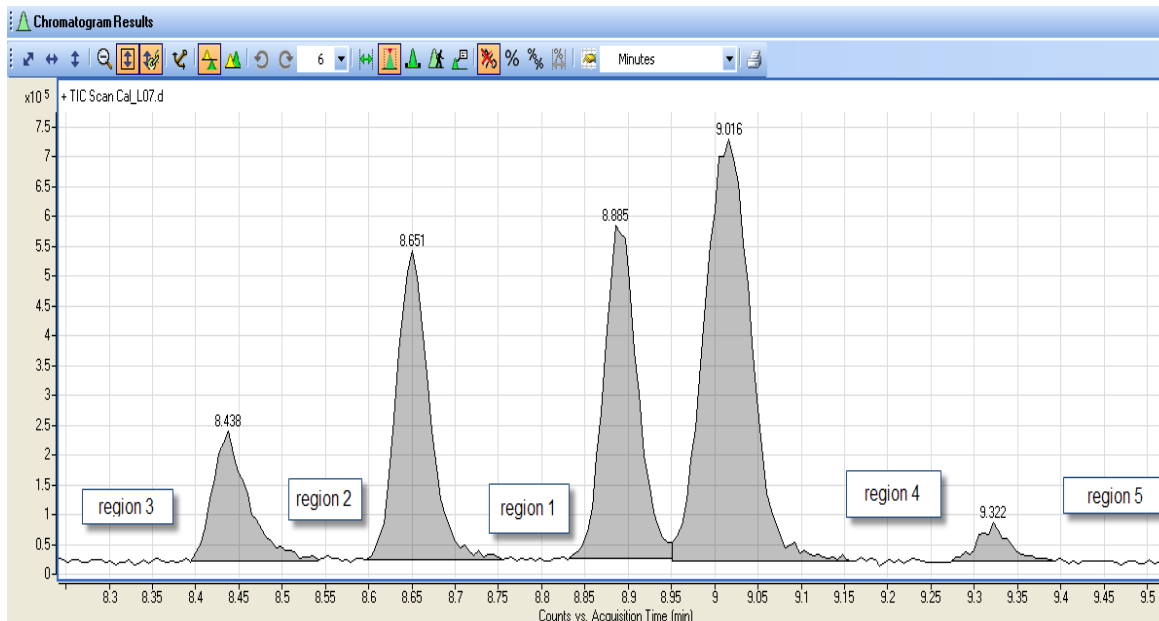
- User defined specific noise regions.
- May be performed automatically when Chromatogram is integrated.



# Calculate Signal-to-Noise

## Automatic Noise Region Detection

- Alternative to user defined specific noise regions in which the software seeks to locate a “noise region” between the peaks found by the integrator
- User specifies a maximum length (desired) and minimum length of noise region and software locates an acceptable region if one exists



The Method Editor window is configured for 'Calculate Signal-to-Noise'. The 'Signal measurement' section has 'Signal definition' set to 'Height'. The 'Noise measurement' section has 'Noise definition' set to 'Peak-to-Peak' with a multiplier of 1. The 'Automatic noise region detection' option is selected, with 'Desired length' set to 1.000 min and 'Minimum length' set to 0.100 min. The 'Specific noise regions' option is unselected, and the 'Automatically compute when chromatograms are integrated' checkbox is also unselected.



Let's take a few moments for questions on Chromatogram Functions.

---

Up Next:  
Training Resources.

# Training Resources

Training resources that are available.

## Convenient Training

Our team of industry experts delivers a quality learning experience with a high degree of flexibility to fit the needs of your lab – in our classrooms, at your site or online:

- **Classroom Training** – Introductory level to in-depth, hands-on training for lab hardware or software.
- **Customized On-Site Training** – Effective learning environment designed to achieve operational excellence and employee development without the need to travel.
- **Online** – From foundation to expert offerings when and where you need it at your own pace



# Introducing Agilent University

## Upgraded customer experience:

- Search and find courses that meet your interests and needs in the format they require

## Introduce new eLearning capabilities:

- Recorded and video-based learning
- Virtual online classes

## Expanded portfolio:

- Foundational subjects
- Intermediate subjects
- Advanced subjects
- Workflow and applications

## Helping customers:

- Educate your employees on Agilent instruments and software
- From new hires to the most seasoned scientists

The screenshot shows the Agilent University website. At the top, the Agilent logo and 'Trusted Answers' are on the left, and navigation links (ABOUT AGILENT, CONTACT US, UNITED STATES, LOGIN) and a search bar are on the right. A red arrow points to the 'TRAINING & EVENTS' menu item. Below the main navigation, a sub-menu for 'Training & Events' is visible, with a red arrow pointing to the 'Education' link. The main content area features a large banner with the text 'AGILENT UNIVERSITY' and a button labeled 'VIEW ALL TRAINING COURSE OFFERINGS >' circled in red. Below the banner, three columns of text describe benefits: 'Increase Tenure and Maximize Productivity', 'Convenient Training', and 'Agilent Training Credits'.

**Campus Pass Key Code Number: CP100OpenLab**

**Good for up to 100 credits**

**Expires 01 Oct2017**

# Questions on today's material...

## Thank you for your attention.



### MassHunter Qualitative Analysis

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